PATENT
Attorney Docket No. 19452A-000320US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

MARTH and ELLIES

Application No.: 09/856,391

Filed: January 7, 2002

For: USE OF CORE 2 GLCNAC TRANSFERASE INHIBITORS IN TREATING INFLAMMATION

Customer No.: 20350

Confirmation No.: 7913

Examiner: Zara, Jane J.

Technology Center/Art Unit: 1635

DECLARATION OF JAMEY MARTH UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Jamey Marth, declare and state as follows:

1. I, Jamey Marth, Ph. D., am a co-inventor of the subject matter of U.S. Patent Application No. 09/856,391, entitled "Use of Core 2 GlcNAc Transferase Inhibitors in Treating Inflammation" (hereinafter "the '391 application" or "the application").

2. I am a Professor of Cellular and Molecular Medicine at the University of California, San Diego, Medical Center, and a Howard Hughes Medical Institute (HHMI) Investigator. I have 25 years of experience in the areas of biology, pharmacology, & immunology. I have a Ph.D. in Pharmacology from The University of Washington, Seattle, WA. A copy of my curriculum vitae is attached hereto as Exhibit C.

3. I have reviewed the Office Action dated November 19, 2004, as well as the Advisory Action dated March 22, 2005. The statements set forth hereinbelow are offered to address the Examiner's remarks in these Actions and to show that the state of the art, pertinent to the pending claims of the '391 application, was sufficiently predictable to allow one of skill in the art to carry out and use the claimed invention without undue experimentation.

4. As of the filing date of the application, it was well-known to those skilled in the art that gene target elimination in null mice is ***very*** predictive of the effects of using inhibitors that interact directly with the respective gene product *in vivo*. Gene ablation had been routinely and extensively utilized to evaluate protein function *in vivo*. Further, because small molecule inhibition of protein function had been a well-established approach for drug development, the pharmaceutical industry had relied on gene ablation studies, including mouse knockouts, to provide insight as to useful protein targets for therapeutic intervention. A comparison of top-selling drugs with data obtained from knockout mice show that there is an excellent correlation between phenotypes in null mice and known drug efficacy. (*See* Zambrowicz *et al.*, *Curr. Opin. Pharmacol.* 3:563-570, 2003 (attached hereto, citing pre-filing date references).)

5. In view of the knowledge in the art summarized above, the skilled artisan reading the '391 application would readily accept that inhibition of C2 GlcNAc transferase protein *in vivo* would provide the effect as recited in the present claims, *inter alia*, inhibition of an inflammatory response in a mammal. The studies set forth in the '391 application established, *inter alia*, modification of leukocyte glycoproteins involved in extravasation of inflammatory cells by C2 GlcNAc transferase and, in particular , inhibition of such extravasation by inhibiting the activity of C2 GlcNAc transferase. Further, these studies identified this enzyme as a particularly advantageous target for specifically modulating the inflammatory response without affecting immune cell function. As shown in the application, using the null mutant mouse model, elimination of C2 GlcNAc transferase function *in vivo* results in inhibition of the extravasation of neutrophils and other inflammatory cells, while leaving lymphocyte trafficking and other immune functions relatively intact (*see* specification at page 18, lines 6-11).

6. In light of the '391 application's teachings, those of skill in the art would readily accept that inhibitors of C2 GlcNAc transferase inhibitors could be used to achieve reduction of an inflammatory response in a mammal. The art of glycosyltransferase inhibitors, including the making and testing of such inhibitors, was well-advanced as of the effective filing date of the application. Further, glycosyltransferase inhibitors had a history of use *in vivo*. Thus, the knowledge in the art of glycosyltransferase inhibitors, including their use *in vitro* and *in vivo*, could be readily applied by the skilled artisan to use C2 GlcNAc transferase inhibitors in carrying out the method as claimed.

7. The skilled artisan would not reasonably view Lowe *et al.* (reference AI in Applicants' IDS) as indicating unpredictability of in vivo use of C2 GlcNAc transferase inhibitors to carry out the claimed method. The language from Lowe *et al.*, cited by the Examiner in the Office Action dated 11/19/2005, relates to the use of inhibitors of the interaction of selectins and their carbohydrate ligands, ***not*** inhibitors of glycosyltransferases. This is an important distinction in the present case. As of the effective filing date, therapeutic approaches based on the direct blocking of receptor-ligand interactions was relatively new, especially with respect to the use of smaller inhibitory molecules (as opposed to, *e.g.*, antibodies or soluble forms of receptors). In contrast, enzymes, including glycosyltransferases, have consistently proven to be very successful drug targets. In particular, the inhibition of enzyme activity via the use of molecules that interact directly with the enzyme protein was a well-established approach in the pharmaceutical industry.

8. With respect to the statement in Lowe *et al.* of a "paucity of information concerning the functional relevance of selectin-dependent leukocyte recruitment in chronic inflammatory conditions," the '391 application provides guidance as to such functional relevance. The studies set forth in the application demonstrate the functional relevance of a particular subset of selectin ligands modified by C2 GlcNAc transferase. Using the null mutant mouse model, elimination of C2 GlcNAc transferase function *in vivo* results in inhibition of the extravasation of neutrophils and other inflammatory cells, while leaving lymphocyte trafficking and other

immune functions relatively intact. The skilled artisan reading the '391 application as of the filing date would readily accept the results of these studies as showing the functional relevance of certain selectin ligands, and of C2 GlcNAc transferase in particular, in inflammatory responses.

9. As to the statements in Lowe *et al.* regarding half-life and binding affinity, these statements are made in the context of the intravascular use of small molecules for direct blocking of receptor-ligand interactions. As noted above, this was a relatively new therapeutic approach as of the effective filing date of the '391 application. The use of smaller molecules for blocking extracellular protein-protein interactions in the intravascular milieu presented new technical issues different than those traditionally encountered, and successfully addressed, with the use of smaller inhibitors for inhibition of enzymes intracellularly. The skilled artisan would not expect, *a priori*, the stability or localized concentrations of compounds used extracellularly in the intravascular space to be representative of the stability or localized concentrations of compounds designed and used to act intracellularly. Nor would the skilled artisan expect binding affinities or compound concentrations necessary to directly block receptor-ligand interactions to be representative of affinities or concentrations necessary to inhibit enzymatic activity. Thus, the skilled artisan would understand the compound pharmacokinetics and pharmacodynamics involved with the approach described in Lowe *et al.* to be significantly different than that involved with method as presently claimed.

10. With respect to specificity of inhibitors, the state of the art in making glycosyltransferase inhibitors was advanced at the time of the invention. For example, analogs of glycosyltransferase substrates were routinely used to design inhibitors having specificity for the corresponding glycosyltransferase. Further, the skilled artisan would reasonably expect glycosyltransferase inhibitors with demonstrated specificity *in vitro* to substantially retain such specificity *in vivo*.

11. Therefore, those of skill in the art would not reasonably view Lowe *et al.* as indicating an "unpredictability of in vivo treatments for inflammation using the instantly claimed approach."

12. The prior use of C2 GlcNAc transferase inhibitors for antiviral or antibacterial agents shows that such inhibitors had been used successfully *in vivo* for other indications. In particular, the *in vivo* use of the C2 GlcNAc glysosyltransferase inhibitors for antiviral or antibacterial indications shows that issues relating to *in vivo* stability, binding affinity, and specificity of such inhibitors had been successfully addressed by those skilled in the art before the filing date. In light of the '391 application's disclosure and the previous successful *in vivo* use of C2 GlcNAc transferase inhibitors, the skilled artisan would readily accept that C2 GlcNAc transferase inhibitors would have *in vivo* efficacy in reducing an inflammatory response.

13. The attached press release from April 2003 ("Inflazyme enters into Agreement to Acquire GlycoDesign," Exhibit B) shows that those skilled in the art recognized that use of glycosyltransferase inhibitors *in vivo* for the treatment of inflammation was not entirely unpredictable. It is clear from the press release that GlycoDesign had developed inhibitors of C2 GlcNAc transferase that were a major part of the value obtained by Inflazyme's purchase of GlycoDesign for $12.8 million. The first item identified as a rationale for the purchase is the fact that GlycoDesign has developed novel Core 2 inhibitors. Therefore, at least some portion of the purchase price of the company can be attributed to the value placed on the C2 GlcNAc transferase inhibitors.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize validity of the application or any patent issuing thereon.

Date: June 16, 2005

By: Jamey D Marth

Name: JAMEY D. MARTH

Title: INVESTIGATOR, HOWARD HUGHES MEDICAL INSTITUTE

PROFESSOR, DEPT. OF CELLULAR AND MOLECULAR MEDICINE, UCSD

ELSEVIER

Predicting drug efficacy: knockouts model pipeline drugs of the pharmaceutical industry

Brian P Zambrowicz, C Alexander Turner and Arthur T Sands

One of the major challenges for the pharmaceutical industry is to develop innovative drugs to new targets from the human genome. A systematic approach for target selection could significantly increase the rate of successful new drug development, thereby enhancing industry productivity. It has previously been shown that mouse knockout phenotypes for the targets of the 100 best-selling pharmaceutical drugs correlate well with known drug efficacy. Furthermore, physiological validation of novel pipeline targets of the pharmaceutical industry has been provided using mouse knockout data. These data demonstrate an excellent correlation between knockout phenotype and anticipated drug efficacy, establishing an important marker for superior new drug targets from the genome.

Addresses
Lexicon Genetics Incorporated, 8800 Technology Forest Place, The Woodlands, TX 77381, USA
Correspondence: Brian Zambrowicz; e-mail: brian@lexgen.com

Current Opinion in Pharmacology 2003, 3:563–570

This review comes from a themed issue on New technologies
Edited by Steven J Projan

1471-4892/$ – see front matter

DOI 10.1016/j.coph.2003.04.002

Abbreviations

ACAT	acyl-CoA cholesterol acyltransferase
COPD	chronic obstructive pulmonary disease
CTLA4	cytotoxic T lymphocyte antigen 4
EDG	endothelial differentiation gene
EGF	epidermal growth factor
Glp-1	glucagon-like peptide-1
Ig	immunoglobulin
KO	knockout
NK	neurokinin
PKC	protein kinase C
TACE	TNF-α-converting enzyme
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor

Introduction

The commercialization of innovative drugs to new targets is critical for both the advancement of human healthcare and the economic future of the pharmaceutical industry. The worldwide bio-pharmaceutical industry has gained approval of two to three new drugs addressing novel targets each year over the past 10 years [1•]. This number of innovative drugs is dwarfed by the number of 'me too' drugs that address targets for which there already exist marketed drugs (typically 25–30 per year). There is a tendency in the industry to balance novel targets in the portfolio with large numbers of programs focused on targets for which there are already drugs on the market. This is partly because of short term concerns surrounding the perceived increased risk of developing drugs to novel targets, as these targets have not received the ultimate validation of approved and marketed drugs with known efficacy in humans. However, if significant progress is to be made in treating unmet medical needs and if the pharmaceutical industry is to deliver continued economic growth, breakthrough drugs to new targets must be systematically developed and successfully commercialized. Technologies are required which can increase the probability that drugs addressing novel targets will produce the desired efficacy in the clinic, thus maximizing the number of breakthrough therapies available to patients. In this review, we examine pipeline drugs (drugs between phase II clinical trials and FDA approval) currently in clinical trials based on modeling of therapeutic effects using mouse gene knockouts (KOs) of the relevant drug targets.

One of the challenges for the pharmaceutical industry is to define a reproducible path of product innovation, bolstering clinical pipelines and improving research and development productivity. Even when a novel drug is developed to a new target, it is unclear if and how the next novel drug will be brought forward. Patents expire on a predetermined 20-year schedule, and management laments the resulting disruption in revenue while discovery efforts during the preceding two decades struggle to produce innovative replacements. Clearly, sustainable growth requires that a reproducible process of drug invention replaces quixotic hopes of drug discovery.

With the sequencing of the human genome, there is now an unprecedented opportunity to systematically examine the targets of all future drugs of the pharmaceutical industry. Defining gene function and target validation are key steps for moving from a gene sequence to drug development, and reliable approaches are required to predict the pharmaceutical utility of a target before drug development commences. Gene KO technology has proven to be an effective method for modeling drug efficacy and determining the physiological functions of mammalian genes. An examination of the targets of the 100 best-selling drugs of the pharmaceutical industry revealed that, in the vast majority of cases, there is a direct correlation between the KO phenotype and the efficacy of drugs which modulate that specific target [1•]. These data define the qualities of a good drug target, and

indicate that a therapeutic effect observed in a KO model is an excellent marker for success in the clinic. With this in mind, we examined the pipelines of the top 10 pharmaceutical companies for drugs addressing novel host targets in phase 2 clinical trials and beyond [2•]. Here, we discuss the KO phenotypes for those targets and the implications for the future success of the corresponding pipeline drugs. We focus on novel targets for which there have been published mouse KOs. This represents the majority of the pipeline targets, although there are a handful of additional novel targets for which KOs do not exist; such targets cannot be assessed. The KO phenotypes of pipeline drug targets are grouped together on the basis of their potential therapeutic area of utility, and are summarized in Table 1.

Neurology

KO mouse studies provide encouraging data for pipeline targets in neurology. New targets for obesity are of great interest because of the current lack of effective drugs with minimal side effects. The cannabinoid CB_1 receptor is the target of antagonists being developed to treat obesity and nicotine addiction. CB_1 receptor KO animals eat less than their wild type littermate controls [3], indicating a role for these receptors in the regulation of food intake. The KO animals also exhibit a decreased ethanol preference, a decrease in self-administration of morphine, decreased behavioral effects of morphine withdrawal and an absence in the rewarding effect of nicotine, suggesting that the CB_1 receptors may be involved in the addictive effects of these drugs [4–6]. The clear behavioral effects of 'knocking out' the CB_1 receptor support the utility of CB_1 antagonists in the treatment of obesity and smoking addiction, as well as in addictive behaviors associated with consumption of other substances.

Another new target for the treatment of obesity is the 5-hydroxytryptamine $5HT_{2C}$ receptor. Agonists at this receptor are under development to reduce body weight by decreasing food intake. Mice lacking the $5HT_{2C}$ receptor exhibit the opposite effect of the agonist drugs; that is, they are overweight because of increased food intake [7]. A third new target affecting feeding behavior is the cholecystokinin-A CCK_1 receptor. Agonists of this target are being developed to treat obesity. Food and water intake is normal in freely fed CCK_1 KO animals, but administration of cholecystokinin does not result in a decrease of food intake in KO animals as it does in wild-type controls [8]. This suggests that the CCK_1 receptor functions to modify short-term feeding behavior, but is non-essential for the long-term regulation of body weight. It remains to be seen whether chronic treatment with drugs that target this receptor will be effective for decreasing body weight.

Neurokinin (NK) receptors are potential new targets for the treatment of depression, nausea and pain. KO of the NK_1 receptor results in decreased anxiety-related behavior in three separate tests of anxiety (elevated plus maze, non-specific fear and maternal separation-induced ultrasonic vocalization) [9]. KO of Tac1, the gene that encodes substance P and NKA (the ligand for the NK_1 receptor), results in decreases in both depression and anxiety-related behavior. Tac1 null animals display decreased depressive-like behavior, as indicated by reduced immobility time in two assays for depression, the forced swim and tail suspension assays [10]. These animals also show decreased levels of anxiety when measured in the elevated plus maze, non-specific fear and open field assays. NK_1 receptor KO animals also display a decreased response in the second phase of the formalin paw test, decreased behavioral reaction to visceral pain, decreased duration of response to capsaicin injection of the heel and reduced response to von Frey hairs after capsaicin injection into the hindpaw. These tests support the use of NK_1 receptor antagonists for the treatment of anxiety, depression, visceral pain and hyperalgesia. NK_1 receptor antagonists have failed as analgesics and it has been argued [11] that the pharmaceutical industry might not have pursued the pain indications if the KO data were available earlier because it suggests that NK_1 receptor antagonists would not be strong analgesics like morphine. The KO animals show no change in pain response in acute tests, such as the hot plate, and responses to stressful stimuli, such as formalin or capsaicin injection, could be the result of an altered emotional rather than pain response.

Other new targets in the area of pain are the adenosine receptors. Adenosine A_1 receptor KO results in increased sensitivity to pain (hyperalgesia), as observed by a decreased latency to respond in the tail flick assay [12]. A_{2A} receptor KO mice have a decreased response to acute pain in the hot plate and tail flick assays and decreased platelet aggregation [13]. KO of the adenosine A_3 receptor results in mice with decreased heat hyperalgesia after carageenan injection into the paw, caused by a reduction in the inflammatory response [14]. The KO of these potential pain targets correlates well with the anticipated efficacy of the corresponding pipeline drugs. Although adenosine A_1 agonists show promise as analgesics, these compounds also have significant side effects. These side effects might be expected given that the adenosine A_1 KO animals exhibit increased anxiety, increased aggressiveness, high blood pressure, decreased muscle strength and reduction in lifespan [12,15].

Metabolism

Mouse KOs have been extremely informative in the area of metabolism. Glucagon-like peptide-1 (Glp-1) receptor agonists are being developed to treat diabetes, as KO of the Glp-1 receptor indicates a role for this pathway in the regulation of glucose homeostasis. Glp-1 receptor KO animals display decreased glucose clearing after both oral and intraperitoneal glucose challenge [16,17]. This

Table 1

Pipeline drug targets of the pharmaceutical industry.

Target	Compound (company)	Stage of development	Indication	KO phenotype
Central cannabinoid CB_1 receptor (antagonist)	Rimonabant (Sanofi-Synthelabo)	Phase 3 clinical	Nicotine use disorder, obesity, psychosis, schizophrenia, septic shock	Lower food intake [3], absence of rewarding effect of nicotine [6]
5-HT_{2C} receptor (agonist)	BVT-933 (GSK)	Phase 2 clinical	Obesity	Increased food intake, increased body weight [7•]
CCK_1 receptor (agonist)	GI-181771 (GSK)	Phase 2 clinical	Gallstones, obesity	Normal food intake and body weight [8]
NK_1 receptor (antagonist)	TAK-637 (Abbott with Takeda) Emend (Merck)	Registered Accepted by FDA	Emesis, inflammation, irritable bowel syndrome, asthma, depression, anxiety disorder, nausea, pain, colitis, inflammatory bowel disease, migraine, cancer, micturition disorder, Pagets disease Nausea	Decreased anxiety and depression-related behavior [9], decreased visceral pain and decreased hyperalgesia. Ligand KO animals display decreased anxiety and depressive-like behavior [10]
Adenosine A_1 receptor (agonist)	GW-493838 (GSK)	Phase 2 clinical	Pain, migraine	Hyperalgesia [12]
Glp-1 receptor (protein ligand agonist)	Exenatide, GLP-1 (Lilly)	Phase 3 clinical	Obesity, insulin-dependent diabetes, non-insulin-dependent diabetes	Glucose intolerance, poor glucose homeostasis [16,17]
Dipeptidyl peptidase V (antagonist)	LAF-237 (Novartis); P32/98 (Merck)	Phase 2 clinical	Non-insulin-dependent diabetes	Improved response to glucose challenge, improved glucose homeostasis [18]
PKC-β (antagonist)	Lilly	Phase 3 clinical	Diabetic peripheral neuropathy	Improved insulin sensitivity [19], improved glucose homeostasis, improved glucose uptake by cells in response to insulin
ACAT2 (antagonist)	Avasimibe (Parke-Davis and Co; Pfizer)	Phase 3 clinical	Hyperlipidemia, atherosclerosis	Lower cholesterol levels, high cholesterol diet does not result in hypercholesterolemia or cholesterol accumulation in liver [20]
Thromboxane A2 receptor (antagonist)	Ifetroban sodium (BMS)	Phase 2 clinical	Peripheral vascular disease, thromboembolism, ulcer, ischemia	Prolonged bleeding times, decreased platelet aggregation [21]
Vasopeptidase (dual ACE + NEP inhibitor) (antagonist)	Gemopatrilat, omapatrilat (Vanlev; BMS); fasidotril (Lilly); reacecadotril (GSK)	Launched	Hypertension, cardiovascular disease, renovascular hypertension, cardiac failure, diarrhea	Decreased blood pressure [22]
Aldosterone receptor (antagonist)	Eplerenone (Novartis - Pfizer [Pharmacia] is licensee)	Registered	Hypertension, cardiac failure	Renal sodium and water loss, dehydration, pseudoaldosteronism [23]
Vasopressin V_2 receptor (antagonist)	SR-121463 (Sanofi-Synthelabo)	Phase 2 clinical	Glaucoma, congestive heart failure, inappropriate ADH syndrome, hypertension	Decreased urine molalities, unable to concentrate urine [24]
NHE1 (antagonist)	Zoniporide (CP-597396; Pfizer)	Phase 2 clinical	Ischemic heart disease	Retarded growth, ataxia, seizures, stomach pathology [25]
CTLA4 (protein therapeutic)	BMS-188667, BMS-224818 (Bristol-Myers Squibb Co)	Phase 2 clinical	Multiple sclerosis, organ transplantation, psoriasis, rheumatoid arthritis, autoimmune disease, transplant rejection, graft versus host disease	Lymphoproliferative disorder [26]
IgE (monoclonal antibody)	Omalizumab (Xolair; Genentech Inc.); TNX-901 (Roche, with Novartis and Tanox)	Registered	Allergic rhinitis, asthma	IgE receptor null animals have decreased responsiveness in mouse models of asthma [27,28•]
Phosphodiesterase-4 (antagonist)	Roflumilast (Pfizer); AWD-12291 = GW-842470, cilomilast (GSK) PDE4 inhibitor (Merck)	Pre-registration	Chronic obstructive pulmonary disease, asthma, allergic rhinitis, rheumatoid arthritis	Decreased response to lipopolysaccharide, decreased anaphylaxis in response to ovalbumin challenge [29•]

Table 1 Continued

Target	Compound (company)	Stage of development	Indication	KO phenotype
TACE (antagonist)	BMS-561392 (BMS)	Phase 2 clinical	Inflammatory bowel disease, rheumatoid arthritis	Embryonic lethality, T cells release 90% less TNF-α when stimulated with anti-CD3 antibody [32]
α4/β1 integrin (antagonists and monoclonal antibodies)	GW-559090 (GSK); R-411 (Roche)	Registered	Allergic rhinitis, asthma	Embryonic lethality for both α4 and β1 KOs [33,34]
EDG receptors (agonist)	FTY-720 (Novartis)	Phase 2 clinical	Organ transplantation, autoimmune disease	Embryonic lethality, fibroblasts defective in sphingosine-1-phosphate-induced chemotaxis [37]
VEGF receptor (antagonists and monoclonal antibodies)	Pegaptanib, SU-6668 (Pfizer); vatalanib, midostaurine (mixed; Novartis); ZD-6474 (AstraZeneca); ranibizumab and bevacizumab (Roche [opt-in right from Genentech])	Phase 3 clinical	Age-related macular degeneration, diabetic retinopathy, cancer	Both Flt-1 and Flk-1 receptor KOs result in embryonic lethality due to vascular defects and inability to form blood vessels [40,41]. KO of the ligand, VEGF, results in embryonic lethality due to inability to develop blood vessels [38,39]. VEGF null embryonic stem cells are impaired in tumor formation when implanted into nude mice
EGF receptor (antagonist)	GS-572016 (GSK); CI-1033, CP-547632 (Pfizer); erlotinib (Tarceva; Pfizer/Roche); ZD-6474; gefitinib (Iressa; AstraZeneca); cetuximab (Erbitux; BMS)	Launched	Solid tumor, cancer, myeloproliferative disorder	Embryonic lethality with multiple organ defects [42,43]
Keratinocyte growth factor-2 (secreted protein therapeutic)	Repifermin (GSK with HGS)	Phase 2 clinical	Nervous system injury, mucositis, inflammatory bowel disease, ulcerative colitis, wound healing, skin ulcer, Crohns disease	Decreased numbers of proliferating cells in basal layer of skin [44]
Cathepsin K (antagonist)	AAE-581 (Novartis)	Phase 2 clinical	Osteoporosis	Osteopetrosis, defective bone resorption by osteoclasts [45]

This table contains the innovative new targets for which there are currently drugs in development in the top 10 pharmaceutical pipelines. ACE, angiotensin-converting enzyme; ADH, antidiuretic hormone; NEP, neutral endopeptidase; NHE1, Na^+/H^+ exchanger).

glucose intolerance is associated with lower levels of circulating insulin and is observed in both heterozygotes and homozygotes. Glp-1 and glucose-dependent insulinotropic peptide are both substrates of dipeptidyl peptidase, which cleaves and inactivates the two peptides. Dipeptidyl peptidase KO animals have increased levels of Glp-1, resulting in increased insulin secretion and glucose clearance in response to oral glucose challenge [18•]. These data provide strong evidence for the involvement of this pathway in maintaining glucose homeostasis in the treatment of diabetes.

Inhibitors of protein kinase C (PKC)-β are being developed for the treatment of complications of diabetes, such as peripheral neuropathy. It is hypothesized that hyperglycemia leads to PKC activation and subsequent changes in pain perception. KO of PKC-β results in mice with 10% lower blood glucose levels in the fasted state and after intraperitoneal glucose challenge, as well as slightly lower plasma insulin levels [19]. Both adipocytes and muscle cells from KO animals exhibit increased uptake of 2-deoxyglucose in the basal state; adipocytes also show increased 2-deoxyglucose uptake in response to insulin. Although no direct effect on neuronal cell function relating to pain was shown, these data demonstrate a role for PKC-β in glucose homeostasis and a potential use for PKC-β inhibitors in the treatment of diabetic complications through the regulation of blood glucose levels.

Cardiology

Traditionally, KO data have been critical in the understanding of targets in cardiology. Inhibitors of acyl-CoA cholesterol acyltransferase (ACAT) are being developed for the treatment of atherosclerosis and dyslipidemia. ACAT2−/− animals display lower plasma cholesterol levels when fed a high-fat diet, reduced cholesterol ester

concentrations in plasma lipoproteins regardless of diet, a shift toward smaller apolipoprotein-B-containing particles, and decreased intestinal absorption of cholesterol when fed a high-cholesterol diet [20]. Importantly, a high-cholesterol diet does not result in hypercholesterolemia or accumulation of cholesterol in the liver of ACAT2 KO mice. The utility of this target for the treatment of dislipidemia is apparent on the basis of these KO data.

Thromboxane receptor antagonists are being developed to reduce thrombosis by decreasing platelet aggregation. KO of the thromboxane prostanoid receptor results in prolonged bleeding times, decreased platelet aggregation in response to thromboxanes or collagen, resistance to thromboembolism, and slight but non-statistically significant decreases in cystolic blood pressure [21]. These data correlate well with the expected drug efficacy.

Neutral endopeptidase is a protease responsible for cleaving and inactivating peptides in the blood that regulate blood pressure. Inhibitors of this target are being developed to treat hypertension. Animals lacking neutral endopeptidase exhibit a 20% decrease in mean arterial blood pressure and increased basal extravasation compared with control animals [22]. These data indicate that neutral endopeptidase plays a critical role in regulating blood pressure.

The aldosterone receptor is another novel target for antihypertensive drugs. KO of the aldosterone receptor results in mice with symptoms of pseudoaldosteronism [23]. KO animals die about 10 days after birth from dehydration caused by renal sodium and water loss. These data clearly demonstrate the role of the mineralcorticoid receptor in regulating sodium reabsorption by the kidney and in maintaining salt balance and blood pressure [24]. An additional new target for the regulation of salt and water balance in the treatment of hypertension is the vasopressin V_2 receptor. Mutation of this receptor further supports its use in hypertension, as newborn animals carrying a nonsense mutation have decreased basal urine molalities and cannot concentrate urine.

An example of a new target in ischemic heart disease is the Na^+/H^+ exchanger NHE1. The KO phenotype for this target does not correlate well with the proposed use of pipeline drugs targeting this channel. KO animals exhibit retarded growth, ataxia and seizures, as well as pathological abnormalities in the stomach [25].

Immunology

One novel target for the treatment of inflammatory disease is cytotoxic T lymphocyte antigen 4 (CTLA4). Both CTLA4 and CD28 bind the ligand CD80 but exert opposite effects: CD28 enhances T cell responses, whereas they are suppressed by CTLA4. KO of CTLA4 results in death of the animals at 2–3 weeks of age as a result of lymphoproliferative disorder [26]. These data support the hypothesized role of CTLA4 in suppressing the immune response. A fusion protein of CTLA4 and immunoglobulin (Ig), CTLA4Ig, is being developed to treat inflammation by binding CD80 and preventing its pro-inflammatory effects that occur upon interaction with CD28.

Xolair is a monoclonal antibody against IgE being developed to treat asthma. IgE receptor null animals display decreased responsiveness in mouse models of asthma. In the ovalbumin challenge model of asthma, KO animals have lower eosinophil numbers and interleukin-4 levels in their bronchial lavage fluid [27•]. Compared with wild-type controls, KO animals also have reduced airway hyperresponsiveness either when given interleukin-5 antibody before ovalbumin challenge or when ovalbumin-challenged animals are given a subsequent higher dose together with methacholine. In addition, null mutants are resistant to both systemic and cutaneous anaphylaxis [28]. These results indicate the importance of IgE and its receptor in allergy and asthma.

Phosphodiesterase-4 inhibitors are being developed for the treatment of chronic obstructive pulmonary disease (COPD). PDE4 KO results in a 90% decrease in lipopolysaccharide-induced tumor necrosis factor-α (TNF-α) release by circulating leukocytes and other inflammatory cells [29]. Because TNF-α is thought to play a role in a variety of inflammatory processes, such as rheumatoid arthritis and septic shock, the data indicate a use for this target in the treatment of inflammatory diseases. Although it may be possible to address the inflammatory component of COPD using PDE4 inhibitors, it is important to recognize that using an anti-inflammatory mechanism against a complex and longstanding disease process such as COPD is, at best, an indirect strategy of intervention that is likely to face serious challenges.

Inhibitors of TNF-α-converting enzyme (TACE) are being developed for the treatment of inflammatory diseases, such as rheumatoid arthritis. Mutation of TACE results in embryonic lethality, and studies performed using cells lacking TACE indicate that this protease cleaves a variety of cell surface proteins other than TNF-α [30,31]. These data indicate that TACE inhibitors may have a variety of side effects as a result of the broad ectodomain shedding activity of this protease. However, when T lymphocytes lacking TACE are stimulated with anti-CD3 antibody, they release 90% less TNF-α and express more surface TNF-α than do normal T cells, demonstrating a clear role for TACE in the cleavage of TNF-α from the cell surface [32].

Small molecule drugs and antibodies that block the function of α4/β1 integrin are being developed to treat inflammatory disease through the inhibition of lymphocyte trafficking. KO of either α4 [33] or β1 [34] integrin

results in embryonic lethality, making it difficult to test the inflammatory hypothesis in these transgenic animals. Endothelial differentiation gene (EDG) receptor modulators are also being developed to prevent transplant rejection by altering lymphocyte trafficking. These compounds are agonists of the EDG receptors and, in both animal models and humans, they cause an altered distribution of T lymphocytes in the peripheral blood due to sequestration in the lymphoid organs [35,36]. The pipeline drug FTY720 is most potent at EDG-1, followed by EDG-8, with decreasing potency at other EDG receptors. KO animals have been developed for the EDG-1 receptor, but not for EDG-8. EDG-1 KO animals die during embryogenesis from a defect in vascular maturation [37]. This defect might be due to abnormal sphingosine-1-phosphate-induced cell migration, as fibroblasts from mutant embryos are defective in chemotaxis induced by sphingosine 1-phosphate. Although this embryonic lethality makes it impossible to test the effects on lymphocyte migration in adult mice, the defect in fibroblast cell migration might parallel the effects of EDG-1 on lymphocyte trafficking.

Oncology

Antibodies and small molecule inhibitors against vascular endothelial growth factor (VEGF) and the VEGF receptor, respectively, are in development in oncology. The strategy is to attenuate tumor angiogenesis by blocking the VEGF signal. KO of VEGF or its receptors, Flt-1 and Flk-1, clearly indicates the importance of this pathway in angiogenesis. Disruption of one copy of VEGF results in embryonic lethality, with defects in angiogenesis and blood island formation [38,39]. In addition, VEGF null embryonic stem cells display a dramatic reduction in the ability to form tumors in nude mice. Furthermore, KO of either Flk-1 or Flt-1 receptor results in embryonic lethality. Flk-1 null embryos lack blood islands, organized blood vessels are absent from the yolk sac and hematopoietic progenitor cells are greatly reduced [40]. Flt-1 null embryos develop endothelial cells but not organized vasculature, and they display vascular overgrowth [41]. The severe KO phenotypes for both ligand and receptors of this pathway might portend mechanism-based side effects in the hematopoetic system. Nevertheless, the rationale for developing drugs that block the VEGF pathway to prevent vascularization of solid tumors makes sense given the KO data, and a degree of on-target toxicity might be tolerable given the medical indication.

Another oncology target is the epidermal growth factor (EGF) receptor. EGF receptor KO mice die *in utero* or shortly after birth with a variety of defects, indicating the importance of EGF signaling in embryonic development [42,43]. The requirement of EGF signaling for the development of multiple organs poses a challenge when developing EGF receptor agonists because of the array of on-target effects that could result from small molecule modulation of this receptor.

Keratinocyte growth factor-2 is being developed as a therapeutic protein to treat mucositis caused by chemotherapy. KO of this growth factor clearly demonstrates its role in development of the epidermis [44]. Null mutants have decreased proliferating cells in the basal layer of newborn skin, a finding that, although supportive of the therapeutic rationale, is a relatively subtle finding given the desired indication.

Bone

A new target for the treatment of osteoporosis is cathepsin K. Cathepsin K KO animals have osteopetrosis as a result of impaired resorptive ability of osteoclasts [45]. These KO data demonstrate the potential for cathepsin K inhibitors for maintaining bone mineral density through inhibition of bone resorption.

Conclusions

Our analysis of pipeline drugs of the top 10 pharmaceutical companies addressing novel targets reveals that over 85% demonstrate a sound biological rationale for the selected disease indication on the basis of KO phenotypes. If the compounds addressing these targets are potent and specific with minimal side effects, they have an opportunity to reach efficacy endpoints and make it onto the market. This suggests that KO mouse data have become an important criteria for development projects in the pharmaceutical industry — it is no coincidence that KOs have been developed for the vast majority of pipeline drug targets. Although it is impossible to judge those novel targets for which no KOs have been published, it is safe to conclude that the level of uncertainty surrounding such targets increases their risk relative to those targets for which *in vivo* validation has been established.

Gene KOs of approximately 20% of pipeline targets (5 out of 24) result in homozygous lethality, a similar fraction to that for targets of the top-selling drugs [1•]. Three of these (VEGF, VEGF receptor and EGF receptor) are being pursued as targets in oncology, a medical indication for which significant inhibition of tissue-specific cell proliferation is a desirable goal and the lethal phenotype is easily rationalized. In cases where null mutations are homozygous lethal, heterozygous animals should be thoroughly studied for effects of a 50% dose reduction of the target protein. Furthermore, such genes should be further analyzed by conditional KO techniques.

A convincing therapeutic strategy was not directly apparent through gene KOs for only two targets: NHE1 and PDE4. NHE1 KO animals demonstrated multiple developmental defects, making interpretation of the phenotype difficult relative to the medical indication pursued (ischemic heart disease). Although the PDE4 KO did

show an anti-inflammatory phenotype, it is only indirectly related to the multifactorial disease pursued (COPD).

At Lexicon, we have industrialized the systematic approach to KOs and are now analyzing the phenotypes of all druggable classes of genes, which we estimate to be about 5 000. These druggable genes include, firstly, all of the gene families currently considered tractable for small molecule drug discovery; secondly, potential targets for monoclonal antibodies; and finally, secreted proteins that could themselves be therapeutics. We have already completed KO and phenotypic analysis of 1 250 druggable genes, and will finish all 5 000 within the next four years. Using this approach, key switches in mammalian physiology are identified, providing a reliable marker for good targets demonstrated by data both for pipeline targets of the industry presented here and for targets of the best-selling drugs on the market [1•]. This strategy is successfully identifying targets in major therapeutic areas, including neurology, metabolism, immunology, cardiology and oncology. KO technology is a robust method for identifying a steady pipeline of *in vivo* validated targets, and provides a means to fuel reliable product innovation in the pharmaceutical industry.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zambrowicz BP, Sands AT: **Knockouts model the 100 best-selling drugs–will they model the next 100?** *Nat Rev Drug Discov* 2003, **2**:38-51.

This review summarizes the phenotypes for the KOs of the targets of the best-selling drugs of the pharmaceutical industry. There is a strong correlation between knockout phenotypes and known drug efficacy.

2. Editors: **Best Pipelines.** *R&D Directions* 2003 **9**:38-51.

This paper describes 10 of the top pharmaceutical pipelines.

3. Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T *et al.*: **Leptin-regulated endocannabinoids are involved in maintaining food intake.** *Nature* 2001, **410**:822-825.

4. Wang L, Liu J, Harvey-White J, Zimmer A, Kunos G: **Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice.** *Proc Natl Acad Sci USA* 2003, **100**:1393-1398.

5. Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP *et al.*: **Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice.** *Science* 1999, **283**:401-404.

6. Castane A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O: **Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence.** *Neuropharmacology* 2002, **43**:857-867.

7. Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D: **Eating disorder and epilepsy in mice lacking 5-HT2C serotonin receptors.** *Nature* 1995, **374**:542-546.

8. Kopin AS, Mathes WF, McBride EW, Nguyen M, Al-Haider W, Schmitz F, Bonner-Weir S, Kanarek R, Beinborn M: **The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight.** *J Clin Invest* 1999, **103**:383-391.

9. Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P, Hen R, Heath MJ: **Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function.** *Proc Natl Acad Sci USA* 2001, **98**:1912-1917.

10. Bilkei-Gorzo A, Racz I, Michel K, Zimmer A: **Diminished anxiety- and depression-related behaviors in mice with selective deletion of the Tac1 gene.** *J Neurosci* 2002, **22**:10046-10052.

11. Hill R: **NK1 (substance P) receptor antagonists – why are they not analgesic in humans?** *Trends Pharmacol Sci* 2000, **21**:244-246.

12. Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Gimenez-Llort L, Escorihuela RM, Fernandez-Teruel A, Wiesenfeld-Hallin Z, Xu XJ *et al.*: **Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor.** *Proc Natl Acad Sci USA* 2001, **98**:9407-9412.

13. Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, Costentin J, Heath JK, Vassart G, Parmentier M: **Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor.** *Nature* 1997, **388**:674-678.

14. Wu WP, Hao JX, Halldner-Henriksson L, Xu XJ, Jacobson MA, Wiesenfeld-Hallin Z, Fredholm BB: **Decreased inflammatory pain due to reduced carrageenan-induced inflammation in mice lacking adenosine A3 receptors.** *Neuroscience* 2002, **114**:523-527.

15. Gimenez-Llort L, Fernandez-Teruel A, Escorihuela RM, Fredholm BB, Tobena A, Pekny M, Johansson B: **Mice lacking the adenosine A1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate.** *Eur J Neurosci* 2002, **16**:547-550.

16. Scrocchi LA, Brown TJ, Maclusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ: **Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene.** *Nat Med* 1996, **2**:1254-1258.

17. Scrocchi LA, Marshall BA, Cook SM, Brubaker PL, Drucker DJ: **Identification of glucagon-like peptide 1 (GLP-1) actions essential for glucose homeostasis in mice with disruption of GLP-1 receptor signaling.** *Diabetes* 1998, **47**:632-639.

18. Marguet D, Baggio L, Kobayashi T, Bernard AM, Pierres M, Nielsen PF, Ribel U, Watanabe T, Drucker DJ, Wagtmann N: **Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26.** *Proc Natl Acad Sci USA* 2000, **97**:6874-6879.

This paper provides an example of a KO phenotype that helps one hypothesize what the efficacy might be for a small molecule antagonist of the target.

19. Standaert ML, Bandyopadhyay G, Galloway L, Soto J, Ono Y, Kikkawa U, Farese RV, Leitges M: **Effects of knockout of the protein kinase C beta gene on glucose transport and glucose homeostasis.** *Endocrinology* 1999, **140**:4470-4477.

20. Buhman KK, Accad M, Novak S, Choi RS, Wong JS, Hamilton RL, Turley S, Farese RV Jr: **Resistance to diet-induced hypercholesterolemia and gallstone formation in ACAT2-deficient mice.** *Nat Med* 2000, **6**:1341-1347.

21. Thomas DW, Mannon RB, Mannon PJ, Latour A, Oliver JA, Hoffman M, Smithies O, Koller BH, Coffman TM: **Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane A2.** *J Clin Invest* 1998, **102**:1994-2001.

22. Lu B, Figini M, Emanueli C, Geppetti P, Grady EF, Gerard NP, Ansell J, Payan DG, Gerard C, Bunnett N: **The control of microvascular permeability and blood pressure by neutral endopeptidase.** *Nat Med* 1997, **3**:904-907.

23. Berger S, Bleich M, Schmid W, Cole TJ, Peters J, Watanabe H, Kriz W, Warth R, Greger R, Schutz G: **Mineralocorticoid receptor knockout mice: pathophysiology of Na^+ metabolism.** *Proc Natl Acad Sci USA* 1998, **95**:9424-9429.

24. Yun J, Schoneberg T, Liu J, Schulz A, Ecelbarger CA, Promeneur D, Nielsen S, Sheng H, Grinberg A, Deng C *et al.*: **Generation and phenotype of mice harboring a nonsense mutation in the V2 vasopressin receptor gene.** *J Clin Invest* 2000, **106**:1361-1371.

25. Bell SM, Schreiner CM, Schultheis PJ, Miller ML, Evans RL, Vorhees CV, Shull GE, Scott WJ: **Targeted disruption of the murine Nhe1 locus induces ataxia, growth retardation, and seizures.** *Am J Physiol* 1999, **276**:C788-C795.

26. Chambers CA, Sullivan TJ, Allison JP: **Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells.** *Immunity* 1997, **7**:885-895.

27. • Mayr SI, Zuberi RI, Zhang M, de Sousa-Hitzler J, Ngo K, Kuwabara Y, Yu L, Fung-Leung WP, Liu FT: **IgE-dependent mast cell activation potentiates airway responses in murine asthma models.** *J Immunol* 2002, **169**:2061-2068.

This paper provides an example of a KO phenotype that helps one hypothesize what the efficacy might be for an antibody to inhibit or neutralize the target.

28. Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP: **Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene.** *Cell* 1993, **75**:969-976.

29. Jin SL, Conti M: **Induction of the cyclic nucleotide phosphodiesterase PDE4B is essential for LPS-activated TNF-alpha responses.** *Proc Natl Acad Sci USA* 2002, **99**:7628-7633.

30. Zhao J, Chen H, Peschon JJ, Shi W, Zhang Y, Frank SJ, Warburton D: **Pulmonary hypoplasia in mice lacking tumor necrosis factor-alpha converting enzyme indicates an indispensable role for cell surface protein shedding during embryonic lung branching morphogenesis.** *Dev Biol* 2001, **232**:204-218.

31. Peschon JJ, Slack JL, Reddy P, Stocking KL, Sunnarborg SW, Lee DC, Russell WE, Castner BJ, Johnson RS, Fitzner JN *et al.*: **An essential role for ectodomain shedding in mammalian development.** *Science* 1998, **282**:1281-1284.

32. Cerretti DP: **Characterization of the tumour necrosis factor alpha-converting enzyme, TACE/ADAM17.** *Biochem Soc Trans* 1999, **27**:219-223.

33. Arroyo AG, Yang JT, Rayburn H, Hynes RO: **Alpha4 integrins regulate the proliferation/differentiation balance of multilineage hematopoietic progenitors *in vivo*.** *Immunity* 1999, **11**:555-566.

34. Fassler R, Meyer M: **Consequences of lack of beta 1 integrin gene expression in mice.** *Genes Dev* 1995, **9**:1896-1908.

35. Brinkmann V, Pinschewer DD, Feng L, Chen S: **FTY720: altered lymphocyte traffic results in allograft protection.** *Transplantation* 2001, **72**:764-769.

36. Napoli KL: **The FTY720 story.** *Ther Drug Monit* 2000, **22**:47-51.

37. Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, Hobson JP, Rosenfeldt HM, Nava VE, Chae SS, Lee MJ *et al.*: **Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation.** *J Clin Invest* 2000, **106**:951-961.

38. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C *et al.*: **Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele.** *Nature* 1996, **380**:435-439.

39. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW: **Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene.** *Nature* 1996, **380**:439-442.

40. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC: **Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice.** *Nature* 1995, **376**:62-66.

41. Fong GH, Rossant J, Gertsenstein M, Breitman ML: **Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium.** *Nature* 1995, **376**:66-70.

42. Miettinen PJ, Chin JR, Shum L, Slavkin HC, Shuler CF, Derynck R, Werb Z: **Epidermal growth factor receptor function is necessary for normal craniofacial development and palate closure.** *Nat Genet* 1999, **22**:69-73.

43. Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, LaMantia C, Mourton T, Herrup K, Harris RC *et al.*: **Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype.** *Science* 1995, **269**:230-234.

44. Suzuki K, Yamanishi K, Mori O, Kamikawa M, Andersen B, Kato S, Toyoda T, Yamada G: **Defective terminal differentiation and hypoplasia of the epidermis in mice lacking the Fgf10 gene.** *FEBS Lett* 2000, **481**:53-56.

45. Saftig P, Hunziker E, Wehmeyer O, Jones S, Boyde A, Rommerskirch W, Moritz JD, Schu P, von Figura K: **Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice.** *Proc Natl Acad Sci USA* 1998, **95**:13453-13458.

Inflazyme enters into Agreement to Acquire GlycoDesign

Vancouver, B.C. and Toronto, Ontario, Canada, April 9, 2003 --

Inflazyme Pharmaceuticals Ltd. (TSX: IZP) and GlycoDesign Inc. (TSX: GD) today announced that they have entered into a definitive agreement whereby Inflazyme has agreed to acquire GlycoDesign. Inflazyme will issue 22 million shares to acquire all outstanding GlycoDesign shares in a deal valued at approximately $12.8 million (see below). The acquisition will expand Inflazyme's franchise in inflammation and strengthen its product pipeline. The acquisition is subject to approval by GlycoDesign shareholders and regulatory authorities.

Ian McBeath, President & CEO of Inflazyme Pharmaceuticals said today: "The acquisition of GlycoDesign will add further anti-inflammatory technology and expand our pipeline and potential partnering opportunities whilst adding to our cash reserves and capital base. This acquisition is part of our strategy to grow Inflazyme into a leading biopharmaceutical company and to build a franchise in the treatment of inflammation".

Michael Thomas, President & CEO of GlycoDesign today said: "GlycoDesign shareholders will benefit through the receipt of shares in a new, more broadly based Company with a larger product pipeline and greater resources, capable of delivering increased value to all shareholders. The new combined Company will build on its existing drug development capabilities and will be better positioned to advance the scientific programs, particularly in the field of inflammation. Importantly for the GlycoDesign shareholder is the realization of unrecognized value in their underlying investment."

Rationale for the Acquisition:

- The acquisition will provide Inflazyme an opportunity to expand its position as a leader in the development of new LSAID (Leukocyte Selective Anti-Inflammatory Drugs) anti-inflammatory therapies by the addition of GlycoDesign's novel CORE2 inhibitors. CORE2 inhibitors are a different type of LSAID and work through inhibition of an enzyme involved in the trafficking of leukocytes to areas of inflammation. Inflazyme

currently has three other distinct series of LSAIDs from its own research. The CORE2 inhibitors will be additive to and expand Inflazyme's LSAID programs.

- Inflazyme's product pipeline will be further expanded by the addition of GlycoDesign's GH9001, a novel anti-thrombotic (anti-blood clotting) therapy, being developed in collaboration with Leo Pharma of Denmark, and currently in Phase I clinical trials. GlycoDesign has further novel anti-thrombotic technology in ATH, a pharmacological coating for blood-contact materials, currently completing pre-clinical studies.
- The acquisition may provide Inflazyme with increased partnering opportunities through a combination of both companies' technologies.
- Inflazyme's financial position will be strengthened giving Inflazyme the flexibility to extend its cash through to the end of 2005.
- The Inflazyme Board and management team will continue to manage the combined business. Ian McBeath and Dr Walter Lovenberg will continue as CEO and, Chairman of the Board respectively. One representative selected by the GlycoDesign Board will be invited to join the Board of Inflazyme.
- Inflazyme's expertise in clinical development and inflammation research is expected to be strengthened by the addition of key personnel from GlycoDesign. Operations will be consolidated into Inflazyme's Vancouver facility.

Details of the Acquisition:

Inflazyme will issue 22 million common shares, on a share exchange basis, for all of the issued and outstanding shares of GlycoDesign. GlycoDesign shareholders will receive 1.8424 Inflazyme common shares for every GlycoDesign share they own. Following the completion of the acquisition, which is expected to occur in June 2003, GlycoDesign shareholders will hold approximately 27.6 % of Inflazyme.

Based on the 10-day average closing price for Inflazyme's shares of $0.58 on the Toronto Stock Exchange for the 10-day period ended on the business day prior to this announcement, the deal is valued at $12.8 million. This values each GlycoDesign share at $1.07, which represents a premium of 174% over the 10-day average closing price of GlycoDesign's shares of $0.39 for the same period.

As at January 31st, 2003, GlycoDesign had working capital of approximately $17.7 million, which included cash and short-term investments of $18.8 million. As at December 31st, 2002 Inflazyme had working capital of approximately $20.4 million, which included approximately $22 million in cash and short-term investments.

The proposed acquisition has the unanimous support of the directors of both GlycoDesign and Inflazyme. Holders of approximately 34.5% of GlycoDesign's common shares have committed their support and agreed to vote their shares in favour of the acquisition. The Board of Directors of GlycoDesign has received a Fairness Opinion from National Bank Financial, Toronto, stating that the exchange ratio is fair from a financial point of view to the GlycoDesign shareholders. SG Cowen Securities Corporation is acting as advisors to Inflazyme.

A Special Meeting of GlycoDesign Shareholders will be held in Toronto on or about May 28th 2003 for the purpose of considering the transaction. Further information about the acquisition will be in the materials to be mailed to GlycoDesign shareholders.

Details of GlycoDesign:

GlycoDesign is a Toronto based drug discovery and development company developing products in the area of glycobiology to treat diseases such as thrombosis, inflammation and cancer. Its lead product, GH9001, being developed in collaboration with Leo Pharma of Denmark, is currently completing Phase I human clinical trials as a new anti-thrombotic agent. GH9001 represents a combination of a medium molecular weight heparin combined with a fractionated highly sulfated dermatan sulphate, which may have advantages over current anti-thrombotic therapies.

GlycoDesign is also developing ATH (anti-thrombin heparin covalent complex), a novel anti-thrombotic coating for devices such as in-dwelling catheters, heart valves and stents that are in human use. This technology is currently in pre-clinical testing and is expected to reduce the thrombogenic effects seen when non-physiologic materials are in contact with blood.

GlycoDesign's CORE2 inhibitor research is focused on the identification of novel small molecule inhibitors of the enzyme core-2 glycosyl transferase. Inhibition of this enzyme blocks leukocyte adhesion and migration and thus may be a new approach to the treatment of inflammatory diseases. GlycoDesign scientists are currently optimizing a number of molecules that show selective inhibition of this target.

Details of Inflazyme:

Inflazyme is a Vancouver based biopharmaceutical company focused on developing new therapies for the treatment of inflammation and other related diseases. Inflazyme's lead technologies are a range of novel, small molecule LSAIDs (Leukocyte Selective Anti-Inflammatory Drugs) that are being developed for a variety of inflammatory diseases. The

Company is developing three distinct series of LSAIDs - the IPL5, IPL12 and IPL99 series. To date three LSAID molecules, from the IPL5 series, have entered human clinical trials.

The most advanced LSAID molecule, IPL512,602 is currently in development for respiratory diseases in partnership with Aventis Pharma and is expected to enter into Phase II clinical trials in Q2'03. This remains as Inflazyme's highest priority. In November 2002 Aventis agreed to take over all program costs for the development of IPL512,602. At the same time the partnership was expanded by the addition of a new LSAID molecule, from Inflazyme's IPL12 series, as a second potential respiratory product.

Inflazyme has other LSAIDs in clinical and pre-clinical development for other inflammatory diseases that are not included in the Aventis partnership.

Inflazyme has also developed a number of inhibitors of the enzyme phosphodiesterase 4 (PDE4). A lead molecule in this series, IPL455,903, was recently partnered with Helicon Therapeutics Inc. as a potential new treatment for disorders of memory associated with stroke and Alzheimer's disease. Other PDE4 molecules remain in development by Inflazyme.

Conference Call

Inflazyme's President and Chief Executive Officer, Ian McBeath and GlycoDesign's President and Chief Executive Officer, Michael Thomas, will host a conference call to discuss this transaction, on April 9th at 8:30 a.m. EST. Live audio of the conference call will be simultaneously broadcast and made available to members of the news media, investors and the general public via Inflazyme's website at www.inflazyme.com. Audio replay of the conference will be available two hours following the completion of the call via Inflazyme's website, or by dialing 1 866 518 1010 (toll free) or 416 252 1143, until Wednesday, May 7, 2003.

Statements in this news release other than historical information are forward-looking statements subject to risks and uncertainties. Actual results could differ materially depending on factors such as the availability of resources, the timing and effects of regulatory actions, the strength of competition, the outcome of litigation and the effectiveness of patent protection. Additional information regarding risks and uncertainties is set forth in the current Annual Information Form for Inflazyme and GlycoDesign on file with the Canadian Securities Commissions. The Toronto Stock Exchange has not reviewed and does not accept responsibility for the adequacy or accuracy of this information.

Contact:

Inflazyme Pharmaceuticals Ltd.
Ian McBeath, President & CEO
Phone (800) 315-3660/604 279 8511
Fax (604) 279 8711
E-mail: Info@inflazyme.com
Website: www.inflazyme.com

Contact:
GlycoDesign,
Michael Thomas, President & CEO
Phone (416) 593 6027
Fax (416) 593 8988
E-mail: mthomas@glycodesign.com
Website: www.glycodesign.com

Media Contact:
Nancy McHarg
James Hoggan & Associates, Inc.
(604) 739-7500

For more information, please contact:

to top of page

Certain information included in this press release is forward-looking and is subject to important risks and uncertainties. The results or events predicted in these statements may differ materially from actual results or events. Factors which could cause results or events to differ from current expectations include, among other things: the impact of rapid technological and market change; the successful and timely completion of clinical studies; the establishment of corporate alliances; new product development; uncertainties related to the regulatory approval process; stock market volatility; and the ability of GlycoDesign Inc. to recruit and retain qualified employees. For additional information with respect to certain of these and other factors, see the reports filed by GlycoDesign Inc. with the Ontario Securities Commission. GlycoDesign Inc. disclaims any intention or obligation to update or revise any forward-looking statements, whether as a result of new information, future events or otherwise.

CURRICULUM VITAE

June, 2005

Jamey Marth
Investigator, Howard Hughes Medical Institute
Professor, Department of Cellular and Molecular Medicine
9500 Gilman Drive-MC0625
CMM-W Bldg., Rm. 333
University of California San Diego
La Jolla, California 92093

858-534-6526, office; 858-534-4064, lab;
858-534-6724, fax; jmarth@ucsd.edu, e-mail

Born April 5, 1957 in Sarasota, Florida
Married with two children

Education

B.Sc. Biochemistry	University of Oregon, Eugene, Oregon Department of Chemistry, Institute of Molecular Biology	1979-1983
Ph.D. Pharmacology	University of Washington, Seattle, Washington Department of Pharmacology, School of Medicine Advisors: Dr. Edwin G. Krebs and Dr. Roger M. Perlmutter	1983-1987
Postdoctoral Studies in Immunology	University of Washington, Howard Hughes Medical Institute Laboratory of Dr. Roger M. Perlmutter	1987-1988
Postdegree Education	Molecular Embryology of the Mouse Cold Spring Harbor Laboratories, Cold Spring Harbor, New York	1989

Positions Held

Staff Scientist, Department of Molecular Biology, Oncogen Corporation Seattle, Washington	1988
Senior Scientist, Biomedical Research Centre, University of British Columbia Vancouver, Canada	1988-1994
Assistant Professor, Department of Medical Genetics University of British Columbia	1989-1994
Associate Professor, Department of Medical Genetics University of British Columbia	1994-1995
Associate Professor, Department of Medicine Division of Cellular and Molecular Medicine University of California San Diego, La Jolla, California	1995-1999
Assistant Investigator, Howard Hughes Medical Institute University of California, San Diego, La Jolla, California	1995-1998
Associate Investigator, Howard Hughes Medical Institute University of California, San Diego, La Jolla, California	1998-present

Professor, Department of Cellular and Molecular Medicine 1999-present
University of California, San Diego, La Jolla, California
Investigator, Howard Hughes Medical Institute 2002-present
University of California, San Diego, La Jolla, California

Memberships

American Association of Immunologists
American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
International Society of Differentiation
Society for Glycobiology

Awards

National Research Service Award	
National Institute of General Medical Sciences	1986-1988
Scholar award, Medical Research Council of Canada	1991-1996
(relinquished upon relocation to UCSD in 1995)	
Investigator award, Howard Hughes Medical Institute	1995-present

Editorial Service

Editorial Board: *Glycobiology* 1996-present

Ad-hoc reviewer for various journals: 1989-present
Cell, Proc. Natl. Acad. Sci. USA, Nature, Nature Medicine, Nature Genetics Nature Biotechnology, Nature Immunology, Molecular and Cellular Biology, EMBO Journal, Molecular Immunology, Journal of Clinical Investigation, Glycobiology, Journal of Immunology, Cancer Research, Journal of Cell Biology, Biochim. Biophys. Acta., Genomics.

Ad-hoc reviewer of research grant applications: 1989-present
National Institutes of Health USA, National Cancer Institute USA
National Science Foundation USA, American Institute of Biological Sciences
Medical Research Council of Canada, National Cancer Institute of Canada

Teaching and Academic Service

Teaching:

Method and Logic in Cell Biology Research, BIOM240	Spring 2003, 2004
Molecular Glycobiology BMS 222/NS 279/BIOL 236	Spring 1996, 1998, 2000, 2002
Biomedical Sciences Graduate Student Minor Proposition, BMS 296	1996-present
Cell Biology, BMS 210	Fall 1999/2000
Systemic Physiology, BMS 213	Fall 2000
Transgenic Animal Modeling of Physiology and Human Disease, SOM 204	Fall 1996/1998
Glycobiology Weekly Journal Club	Fall 1995-1998
Modern Methods in Cellular and Molecular Pathology, PATH 231	Fall 1995/1996
Modern Techniques in Biomedical Research, MED 260	Fall 1995/1996
Advanced Immunogenetics, MEDG 510/MICRO 502	Fall 1993/1994
Homeobox Genes in Development, MEDG 540	Fall 1991
Transgenic Animals in Genetic and Disease Analysis, MEDG 502	Fall 1990, 1991
Topics in Genomic Imprinting, MEDG 530	Sept-Dec 1990

Services:

Search Committee for Chair of Dept. of Pharmacology, UCSD	Fall 2002-2004
Search Committee for faculty recruitment, Dept. of Biology	Fall 2002-2003
Ad-hoc Member, UCSD Faculty Promotions Committee	2001-present

Organized annual School of Medicine CMM Seminar Series	2000-present
UCSD Dept. of CMM Academic Senate member and alternate,	Fall 1999/2000
Steering Committee, Glycobiology Research and Training Center	1999-2002
Founded and Organized annual San Diego Glycobiology Symposium	1998-2000
Admissions Committee, UCSD BMS Graduate Program	1998-1999
Co-Chaired UCSD BSB Vivarium Renovation Committee	Spring 1998
Interim Leader, Glycobiology Program, UCSD Cancer Center	1996-1997
Member CMM Vivaria Users Committee	1995-present
Established and directed UCSD Gene Targeting Core Facility	1995-1998
Medical School Admissions Committee, University of British Columbia	1993-1994
Faculty representative to Medical Research Council	Feb 1993
Search Committee for Director of the Biomedical Research Centre, UBC	1992-1994

Host Laboratory for Visiting and Sabbatical Faculty:

Harry Matthews	U.C. Davis	1992
Shou Takashima	Tokyo, Japan	2000-2001
Suguru Oguri	Hokaido, Japan	2003-2004

Post-Doctorates Supervised:

Guiseppe Bertoni, D.V.M., supported by a Fellowship from the Swiss Science Foundation. 1989-1991
Subsequent position: Research Professor, Institute of Veterinary Virology, University of Berne, Switzerland

Martina Metzler, Ph.D., supported by a Fellowship (1991-1993) from the Swiss Science Foundation 1991-1995
Subsequent position: Research Associate, the Centre for Molecular Medicine and Therapeutics, Vancouver, British Columbia, Canada

Thierry Hennet, Ph.D., supported by a Fellowship from the Swiss Science Foundation. 1993-1996
Subsequent position: Assistant Professor, Physiological Institute, University of Zürich, Switzerland.

John Priatel, Ph.D., supported by funds from the NIH. 1997-1999
Subsequent position: Postdoctoral Researcher, Dept. of Microbiology and Immunology, University of British Columbia, Vancouver, Canada.

Robert Campbell, Ph.D., M.D., supported by a Fellowship (1993-1996) from the Medical ResearchCouncil of Canada. 1993-1998
Subsequent position: Founder, Big Bench Software Co., Vancouver, B.C.

Raheel Shafi, M.D., Ph.D., supported by the HHMI. 1996-1999
Subsequent position: Associate Professor of Pathology, Shifa Medical College and International Hospital, Islamabad, Pakistan

Lesley Ellies, Ph.D., D.D.S., supported by grant funds from the NIH, and an NIH Fellowship (1998-2000). 1995-2000
Subsequent position: Assistant Research Scientist University of California, San Diego, Cancer Center

Kevin Richardson, Ph.D., supported by funds from the NIH. 1999-2000
Subsequent position: Technical Sales Representative, Stratagene, San Diego, California.

Yan Wang, Ph.D., supported by funds from the NIH. 1998-2002
Subsequent position: Scientist, Apovia, Inc., San Diego, California

Niall O-Donnell, Ph.D., supported by the HHMI 1999-2002
Subsequent position: Postdoctoral Fellow, Drug Discovery Immunology Team, Johnson & Johnson Pharmaceutical Research and Development,

- San Diego, California

Kazuaki Ohtsubo, Ph.D., supported by funds from the NIH and the HHMI.	2000-present
Pam Grewal, Ph.D., supported by funds from the NIH and HHMI.	2003-present
Mari Tenno, Ph.D., supported by funds from the NIH and HHMI.	2003-present

Graduate Students Supervised:

Christopher J. Ong,. Ph.D. awarded. Supported by 1989-1995
Medical Research Council student fellowship, 1990-1993
Subsequent positions: Post-doctoral Fellow, University of British Columbia,
Assistant Professor, Dept. of Surgery, University of British Columbia.

Jerry Hendry,. M.Sc. awarded. Supported by grant funds from 1992-1995
MRC and Ciba-Geigy. Subsequent position:
Research Technician, University of British Columbia.
Medical School Student, University of British Columbia.

John J. Priatel, Ph.D.. Ph.D. awarded. Supported by 1990-1997
Roman Babicki UBC student fellowship, 1992-1994
and UCSD 1995-1997. Subsequent position:
Post-doctoral Fellow, University of British Columbia.

Zhengyi Ye, graduate student in Biomedical Sciences, UCSD 1996-2004
Supported by HHMI and NIH research grant funds.
Subsequent position

Steven Van Dyken, graduate student in Biomedical Sciences 2001-present
UCSD. Supported by NIH research grant funds.

Graduate Student Thesis Committees at UCSD: 1995-present
Amena Rahman (Larry Goldstein, thesis advisor)
Murli Krishna (Ajit Varki, thesis advisor)
Trudy Christiansen (Martin Haas, thesis advisor)
Huan-You Wang (Xiang-Dong Fu, thesis advisor)
Frances Putkey (Don Cleveland, thesis advisor)
James Li (K.C. Nicolaou, thesis advisor)
Jennifer Matsuda (Mitchell Kronenberg, thesis advisor)
Helen Chen (Ajit Varki, thesis advisor)
Denise M. Gangadharan (Mitchell Kronenberg, thesis advisor)

Lecture Invitations Accepted

1987

Genentech, San Francisco, California
Du Pont C.R.&D., Glenolden, Pennsylvania.
13th Annual Salk/Seattle Symposium, Friday Harbor, San Juan Islands, Washington.

1988

Virus-Cell Interaction, Heinrich Pette Institut, Hamburg, Germany.
Institut de Pathologie Moleculaire, Paris, France.
Immunex Corporation, Seattle, Washington.

1991

University of Alberta, Edmonton, Alberta, Canada.
University of Toronto, Toronto, Ontario, Canada.
Department of Histology, University of Romè, Rome, Italy.
University of Zürich, Zürich, Switzerland.
Institute of Veterinary Virology, Bern, Switzerland.
"Mouse Molecular Genetics" Conference, Heidelberg, Germany
Signal Transduction in normal and Cancer Cells, Banff Centre, Banff, Alberta, Canada.

1992

The Hospital for Sick Children, Toronto, Ontario, Canada.
18th Annual Salk/Seattle Symposium, Salk Institute, San Diego, California.
Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, U.K.
8th International Congress of Immunology, Budapest, Hungary.
AAAS Science Innovation Symposium, San Francisco, California.
Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, Washington.

1993

The University of Michigan, Ann Arbor, Michigan.
Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, Washington.
Fred Hutchinson Cancer Research Center, Seattle, Washington.
Institute for Allergy & Immunology, La Jolla, California.
Rinsho-Ken International Conference "Frontiers in Glycobiology", Tokyo, Japan.
Ciba-Geigy, Basel, Switzerland.
Imperial Cancer Research Fund, Tumor Immunology Unit, University College London Medical School, London, U.K.
University of Toronto, Toronto, Ontario, Canada.
ICOS Corporation, Bothell, Washington.
Immunex Corporation, Seattle, Washington.

1994

Neuro-Oncology Conference, Chicago Institute for NeuroSurgery and NeuroResearch Chicago, Illinois.
Keystone Symposia on Molecular and Cellular Biology
"Complex Carbohydrates in Biology and Medicine", Frisco, Colorado.
McGill Cancer Research Centre, Montreal, Quebec, Canada.
The Biomembrane Institute, Seattle, Washington.
Fifth International Conference "Lymphocyte Activation and Immune Regulation", University of California, Irvine, California.

1995

13th International Symposium on Glycoconjugates, Seattle, Washington.
Symposium on Glycobiology, San Rosario, Orcas Island, San Juan Islands, Washington.
Regional Primate Research Center, Beaverton, Oregon.
Genentech, San Francisco, California.
Gordon Research Conference, "Glycoproteins and Glycolipids," Oxnard, California.

1996

Mid-Atlantic Developmental Biology Conference, National Institutes of Health, Bethesda, Maryland.
International Symposium: "Molecular and Cell Biology of Glycoconjugate Expression", Zürich, Switzerland.

1997

Society for Glycobiology Symposium, Long Beach, California.
International Conference: "Glycoconjugates and Matrix Molecules in Health and Disease", National Institutes of Health, Bethesda, Maryland.
17th International Congress of IUBMB/ASBMB Symposium "Glycobiology",
San Francisco, California.
Organized and presented Mini-Symposium at 17th International Congress of IUBMB/ASBMB entitled "Transgenics and Conditional Gene Mutagenesis",San Francisco, California.
National Center for Human Genome Research, Bethesda, Maryland.
Department of Dental Research, University of Rochester, Rochester, New York.

CCRC, University of Georgia, Athens, Georgia.
Department of Pharmacology, University of Washington, Seattle, Washington.
West Coast Regional Developmental Biology Conference, Lake Arrowhead, University of California, Los Angeles.
Gordon Research Conference, "Glycobiology", Ventura, California.

1998

19th International Carbohydrate Symposium, San Diego, California.
College of Medicine HHMI Seminar Series, University of Iowa.
International Symposium "Sialobiology and Other Novel Forms of Glycosylation," Taipei, Taiwan.
16th Biotechnology Symposium, Tokyo, Japan.

1999

15th International Symposium on Glycoconjugates, Tokyo, Japan.
Gordon Research Conference "Glycobiology", Ventura, California.
Society for Glycobiology Symposium, San Francisco, California.
Kirin Biotechnology Institute, Yokohama, Japan.

2000

2nd International Symposium: "Glycosyltransferases", Toronto, Ontario.
University of Colorado Health Sciences Center, Denver, Colorado.
GLIBS Glycobiology Symposium, Karolinska Institute, Stockhom, Sweden.
Biochemical Society and British Society for Developmental Biology Symposium Glycobiology of Development", University of Sussex, Brighton, England.

2001

Keystone Symposium Workshop "Regulation of Immunity and Autoimmunity", Keystone, Colorado.
Gordon Research Conference: "Glycobiology", Ventura, California.
7th National Symposium: "Basic Aspects of Vaccines", Uniformed Services University of the Health Sciences, Betheseda, Maryland.
International Symposium: "Protein Traffic, Glycosylation, and Health", Interlaken, Switzerland.
4th Carbohydrate Engineering Meeting, Royal Institute of Technology, Stockholm, Sweden.
Department of Immunology, The Scripps Research Institute, La Jolla, California.
Society for Glycobiology Symposium, San Francisco, California.

2002

San Diego Glycobiology Symposium, San Diego, California.
Department of Immunology, University of Washington, Seattle, Washington.
Massachusetts Institute of Technology, Boston, Massachusetts
Keystone Symposium: "Molecular Mechanisms of Leukocyte Trafficking", Steamboat Springs, Colorado.
3rd International Symposium on Glycosyltransferases, Djuonaset, Stockholm.
3rd Ringberg meeting on "Molecular Mechanisms of Leukocyte Traffic", Ringberg Castle, Germany.

2003

Department of Microbiology and Immunology, University of California, San Francisco, California.
Department of Immunology, Genentech, South San Franscisco, California.
Gordon Research Conference: "Glycobiology", Ventura, California.
ASBMB Experimental Biology Symposium: "Glycobiology",San Diego, California.

Department of Cell Biology, Albert Einstein College of Medicine, New York, New York.
Departments of Physiology and Biochemistry, University of South Florida, Tampa, Florida.
Department of Biochemistry, CCRC, University of Georgia, Athens, Georgia.
Department of Immunology, The Scripps Research Institute, La Jolla, California.

2004

Life Sciences Institute, University of Michigan Medical School, Ann Arbor, Michigan.
4th International "Sialobiology" Conference. St. Andrews University, Scotland.
Symposium on Functional Glycomics, Kazusa Academia Park, Kisarazu, Chiba, Japan.
Joint Meeting of the Japanese and American Consortia for Glycomics, Honolulu, Hawaii.

2005

Glycomics and Carbohydrates in Drug Development, Cambridge Healthtech Institute's 3rd Annual Conference, La Jolla, California.
Institute of Physiology, University of Zurich, Zurich, Switzerland.
Max-Planck Institute for Immunobiology, Freiburg, Germany.
Society for Glycobiology Symposium, Boston, Massachusetts.

Extramural and Professional Activities

Consultant, Transgenic Animal Technologies 1992-1994
Bristol Myers Squibb Pharmaceutical Research Institute, Seattle, Washington

Consultant, Function of Bioactive Carbohydrates and 1998-1999
Glycosyltransferase Inhibition, CYTEL Corporation, San Diego, California

Co-Founder and Chair of the Scientific Advisory Board 1999-present
Abaron Biosciences, Inc., Del Mar, California

Scientific Advisory Board, Xenogen Corporation 2000-present
Alameda, California

Board of Directors, Society for Glycobiology 2002-present

Patents

"Transgene Recombination System: Cre-loxP mutagenesis in transgenic mammals."
Jamey D. Marth, inventor.
Filed July 1992
Status: Abandoned by the University of British Columbia, August 1993.

"Control of Immune Responses by Modulating the Activity of Glycosyltransferases."
Jamey D. Marth and James C. Paulson, inventors.
Filed May 1998
Status: Granted April 2002 #6376475.

"Use of Core 2 GlcNAc Transferase Inhibitors in Treating Inflammation."
Jamey D. Marth and Lesley Ellies, inventors.
Filed November 1998
Status: In Prosecution.

"Diagnosis of Human Glycosylation Disorders."
Jamey D. Marth and Hudson Freeze, inventors.
Filed December 1998
Status: In Prosecution.

"Prevention of Atherosclerosis and Undesired Blood Clotting by Reducing Von Willebrand Factor"
Jamey D. Marth and Lesley Ellies, inventors.
Filed September 1999
Status: In Prosecution.

"Blocking Inflammation by Inhibiting Sialylation."
Jamey D. Marth and Lesley Ellies, inventors.
Filed November 2000
Status: In Prosecution.

"Anxiolytic Treatment by Inhibition of ST8Sia-II Sialyltransferase"
Jamey D. Marth and Minoru Fukuda, inventors.

Provisional Filed February, 2004.

"Control of Glucose Transport and Insulin Secretion by the Mgat4a/b Glycosyltransferase"
Jamey D. Marth and Kauzaki Ohtsubo, inventors.
Provisional Filed May, 2004.

"Treatment of Epilepsy by Up-Regulating the Expression of ST3Gal-III"
Jamey D. Marth, inventor
Provisional Filed July, 2004.

Funding Sources and Direct Costs Awarded

Medical Research Council of Canada, grant entitled 1990-1992
"Hematopoietic Gene Function in Transgenic Mice."
($210,000 total award)

National Centres of Excellence Genetic Basis of Human Disease Program 1990-1994
P.I. of component entitled: "Gene-Targeting Core Facility."
($352,000 total award)

Medical Research Council of Canada Scholarship award entitled 1991-1996
"Hemopoiesis in Transgenic Mice."
($245,000 total award, relinquished upon relocation in 1995)

National Cancer Institute of Canada, grant entitled: 1992-1995
"Immunology in Genetically Defined ES Cells and Transgenic Mice."
($280,158 total award, relinquished upon relocation in 1995)

Medical Research Council of Canada, entitled "Lymphopoiesis in Transgenic Mice." 1993-1996
($288,000 total award, relinquished upon relocation in 1995)

CIBA-Geigy grant, entitled: "Tissue-Specific Ablation of the *lck* and 1994-1996
zap-70 Genes in Transgenic Mice."
($136,800 total award, relinquished upon relocation in 1995)

National Cancer Institute of Canada, entitled: 1994-1997
"Retinoblastoma Gene Involvement in Breast Cancer."
($278,250 total award, relinquished upon relocation in 1995)

National Centres of Excellence: Protein Engineering Program 1994-1998
Component entitled "Role of Complex N-Glycans in Oncogenesis."
($134,000 total award, relinquished upon relocation in 1995)

National Centres of Excellence: "Genetic Basis of Human Disease" Program 1994-1998
Project entitled: "Structure/Function Analyses of the Cre Site-Specific
DNA Recombinase."
($240,000 total award, relinquished upon relocation in 1995)

National Institutes of Health - NIDDK R01 entitled 1994-2007
"Glycosyltransferase Function in Development and Disease"
($1,088,461 total award, presently $172,000/year)

Howard Hughes Medical Institute Investigator Award 1995-2007
"Protein Glycosylation in Mammals."
(funding determined annually, presently ~$810,000/year)

National Institutes of Health - NHLBI Program Project Grant (PPG) entitled: 1997-2007
"Genetic Modulation of Blood and Vascular Glycosylation"
A. Varki, Program P.I.
J. Marth P.I. on PPG project component entitled
"Sialyltransferases in Development and Physiology"
(total award: $902,759, presently $103,000/year)

J. Marth P.I. on PPG component entitled:
"Immune Development and Response Core Facility"
($620,144 total award, presently $58,000/year)
J. Marth co-P.I. on PPG component entitled:
"Mouse Genetics Core Facility"
($2,423,536 total award, presently $86,000/year to co-PI)

National Institutes of Health – National Institute of General Medical Sciences 2001-2006
'Glue Grant' entitled "Consortium for Functional Glycomics."
(J. Paulson, Program P.I,)
J. Marth P.I. on Consortium component entitled "Mouse Phenotype Core Facility"
($6,099,744 total award, presently $777,346/year)

National Institutes of Health – National Institute of General Medical Sciences 2004-2009
Program Project Grant entitled:
"Cell Adhesion Mechanisms in Vascular Disease and Thrombosis"
M. Ginsberg, Program PI
J. Marth P.I. on "Mouse Genetic and Embryonic Stem Cell Facility"
($132,000/year)

Publications

Peer-Reviewed:

1. Marth, J.D., Peet, R., Krebs, E.G., and Perlmutter, R.M. (1985). A lymphocyte-specific protein-tyrosine kinase gene is rearranged and overexpressed in the murine T cell lymphoma LSTRA. *Cell* 43, 393-404.

2. Marth, J.D., Disteche, C., Pravtcheva, D., Ruddle, F., Krebs, E.G., and Perlmutter, R.M. (1986). Localization of a lymphocyte-specific protein tyrosine kinase gene *(lck)* at a site of frequent chromosomal abnormalities in human lymphomas. *Proc. Natl. Acad. Sci. USA* 83, 7400-7404.

3. Ziegler, S.F., Marth, J.D., Lewis, D.B., and Perlmutter, R.M. (1987). Novel protein tyrosine kinase gene (*hck*) preferentially expressed in cells of hematopoietic origin. *Mol. Cell. Biol.* 7, 2276-2285.

4. Marth, J.D., Lewis, D.B., Gearn, M.E., Krebs, E.G., Wilson, C.B., and Perlmutter, R.M. (1987). Regulation of p56lck during T cell activation: Functional implications for the *src*-like protein tyrosine kinases. *EMBO J.* 6, 2727-2734.

5. Marth, J.D., Cooper, J.A., King, C.S., Ziegler, S.F., Tinker, D.A., Overell, R.W., Krebs, E.G., and Perlmutter, R.M. (1988). Neoplastic transformation induced by an activated lymphocyte-specific protein tyrosine kinase (p56lck). *Mol. Cell. Biol.* 8, 540-550.

6. Marth, J.D., Overell, R.W., Meier, K.E., Krebs, E.G., and Perlmutter, R.M. (1988). Translational activation of the *lck* proto-oncogene. *Nature* 332, 171-173.

7. Garvin, A.M., Pawar, S., Marth, J.D., Ziegler, S.F., and Perlmutter, R.M. (1988). Structure of the murine *lck* gene and its rearrangement in a murine lymphoma cell line. *Mol. Cell. Biol.* 8, 3058-3064.

8. Louie, R.R., MacAuley, A., King, C.S., Marth, J.D., Perlmutter, R.M., Eckhart, W., and Cooper, J.A. (1988). p56*lck* protein tyrosine kinase is cytoskeletal and does not bind to polyoma virus middle T antigen. *J. Virol.* 62, 4673-4679.

9. Perlmutter, R.M., Marth, J.D., Lewis, D.B., Peet, R., Ziegler, S.F., and Wilson, C.B. (1988). Structure and expression of *lck* transcripts in human lymphoid cells. *J. Cell. Biochem.* 38, 117-126.

10. Marth, J.D., Lewis, D.B., Cooke, M.P., Mellins, E.D., Gearn, M.E., Samelson, L.E., Wilson, C.B., Miller, A.D., and Perlmutter, R.M. (1989). Lymphocyte activation provokes modification of a lymphocyte specific protein tyrosine kinase (p56lck) *J. Immunol.* 142, 2430-2437.

11. Abraham, K.M., Levin, S.D., Marth, J.D., Forbush, K.A., and Perlmutter, R.M. (1991). Thymic tumorigenesis induced by overexpression of p56lck..

Proc. Natl. Acad. Sci. USA 88, 3977-3981.

12. Abraham, K.M., Levin, S.D., Marth, J.D., Forbush, K.A., and Perlmutter, R.M. (1991). Delayed thymocyte development induced by augmented expression of p56lck. *J. Exp. Med.* 173, 1421-1432.

13. Watts, J.D., Wilson, G.M., Ettehadieh, E., Kubanek, C.-A., Astell, C.R., Marth, J.D., and Aebersold, R. (1992). Purification and initial characterization of the lymphocyte-specific protein tyrosine kinase p56*lck* from a baculovirus expression system. *J. Biol. Chem.* 267, 901-907.

14. Pownall, S., Kozak, C. A., Schappert, K., Sarkar, M., Hull, E., Schachter, H., and Marth, J.D. (1992). Molecular cloning and characterization of the mouse UDP-*N*-acetylglucosamine: α-3-D-mannoside β1,2-*N*-acetylglucosaminyltransferase I gene. *Genomics* 12, 699-704.

15. Orban, P.C., Chui, D., and Marth, J.D. (1992). Tissue- and site-specific DNA recombination in transgenic mice. *Proc. Natl. Acad. Sci. USA* 89, 6861-6865.

16. Chui, D., Ong, C.J., Johnson, P., Teh, H.-S., and Marth, J.D. (1994). Specific CD45 isoforms differentially regulate T cell receptor signaling. *EMBO J.* 13, 798-807.

17. Ong, C.J., Chui, D., Teh, H.-S., and Marth, J.D. (1994). Thymic CD45 tyrosine phosphatase regulates apoptosis and MHC-restricted negative selection. *J. Immunol.* 152, 3793-3805.

18. Metzler, M., Gertz, A., Sarkar, M., Schachter, H., Schrader, J.W., and Marth, J.D. (1994). Complex asparagine-linked oligosaccharides are required for morphogenic events during post-implantation development. *EMBO J.* 13, 2056-2065.

19. Gu, H., Marth, J.D., Orban, P.C., Mossmann, H., and Rajewsky, K. (1994). Deletion of the DNA polymerase beta gene in T cells using tissue-specific gene targeting. *Science* 265, 103-106.

20. Carlow, D.A., Marth, J.D., Clark-Lewis, I., and Teh, H.-S. (1995). Isolation of a gene encoding a developmentally-regulated T cell-specific protein with a guanine nucleotide triphosphate-binding motif. *J. Immunol.* 154, 1724-1734.

21. Dutz, J., Ong, C.J., Marth, J.D., and Teh, H.-S. (1995). Distinct differentiative stages of CD4+CD8+ thymocyte development defined by the lack of coreceptor binding in positive selection. *J. Immunol.* 154, 2588-2599.

22. Nasir, J., Floresco, S.B., O'Kusky, J.R., Diewert, V.M., Richman, J.M., Zeisler, J., Borowski, A., Marth, J.D., Phillips, A.G., and Hayden, M.R. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell* 81, 811-823.

23. Campbell, R.M., Metzler, M., Granowski, M., Dennis, J.W., and Marth, J.D. (1995). Complex asparagine-linked oligosaccharides in *Mgat1*-null embryos. *Glycobiology* 5, 535-543.

24. Lewis, M.E., Forsythe, I.J., Marth, J.D., Brunzell, J.D., Hayden, M.R., and Humphries, R.K. (1995). Retroviral-mediated gene transfer and expression of human lipoprotein lipase (LPL) in somatic cells. *Hum. Gene Therapy* 6, 853-863.

25. Hennet, T., Hagen, F.K., Tabak, L.A., and Marth, J.D. (1995). T cell-specific deletion of a polypeptide N-acetylgalactosaminyltransferase gene by site-directed recombination. *Proc. Natl. Acad. Sci. USA* 92, 12070-12074.

26. Granovsky, M., Fode, C., Warren, C.E., Campbell, R.M., Marth, J.D., Pierce, M., Fregien, N., and Dennis, J.W. (1995). GlcNAc-transferase V and Core 2 GlcNAc-transferase expression in the developing mouse embryo. *Glycobiology* 5, 797-806.

27. Phaneuf, D., Wakamatsu, N., Huang, J.-Q., Borowski, A., Peterson, A.C., Fortunato, S.R., Ritter, G., Igdoura, S.A., Morales, C.R., Benoit, G., Akerman, B.R., Leclerc, D., Hanai, N., Marth, J.D., Trasler, J.M., and Gravel, R.A. (1996). Dramatically different phenotypes in mouse models of Human Tay Sachs and Sandhoff diseases. *Hum. Mol. Gen.* 5, 1-14.

28. Zacksenhaus, E., Jiang, Z., Chung, D., Marth, J.D., Phillips, R.A., and Gallie, B.L. (1996). pRb controls proliferation, differentiation, and death of skeletal muscle cells and other lineages during embryogenesis. *Genes and Development* 10, 3051-3064.

29. Priatel, J.J., Sarkar, M., Schachter, H., and Marth, J.D. (1997). Isolation, characterization, and inactivation of the mouse *Mgat3* gene: The bisecting *N*-acetylglucosamine in asparagine-linked oligosaccharides appears dispensable for viability and reproduction. *Glycobiology* 7, 45-56.

30. Kozieradzki, I., Kündig, T., Kishihara, K., Ong, C.J., Chui, D., Wallace, V.A., Kawai, K., Timms, E., Ionescu, J., Ohashi, P., Marth, J.D., Mak, T.W., and Penninger, J.M. (1997). T cell development in mice expressing splice variants of the protein tyrosine phosphatase CD45. *J. Immunol.* 158, 3130-3139.

31. Ong, C.J., Dutz, J.P., Chui, D., Teh, H.-S., and Marth, J.D. (1997). CD45 enhances positive selection and is expressed at a high level in large, cycling, positively-selected CD4+CD8+ thymocytes. *Immunology* 91, 95-103.

32. Chui, D., Oh-Eda, M., Liao, Y.-F., Panneerselvam, K., Lal, A., Marek, K.W., Freeze, H.H., Moremen, K.W., Fukuda, M.N., and Marth, J.D. (1997). Alpha-mannosidase-II deficiency results in dyserythropoiesis and unveils an alternate pathway in oligosaccharide biosynthesis. *Cell* 90, 157-167.

33. Hennet, T., Chui, D., Paulson, J.C., and Marth, J.D. (1998). Immune regulation by the ST6Gal sialyltransferase. *Proc. Natl. Acad. Sci. USA* 95, 4504-4509.

34. Boyd, R., Kozieradzki, I., Chidgey, A., Mittrucker, H.W., Bouchard, D., Timms, E., Kishihara, K., Ong, C.J., Chui, D., Marth, J.D., Mak, T.W., and Penninger, J.M. (1998).
Receptor-specific allelic exclusion of TCR-V alpha-chains during development.
J. Immunol. 161, 1718-1727.

35. Wang, S.P., Marth, J.D., Oligny, L., Vachon, M., Robert, M.-F., Ashmarina, L., and Mitchell, G.A. (1998). 3-Hydroxy-3-Methylglutaryl-CoA lyase (HL):
Gene targeting causes prenatal lethality in HL deficient mice.
Hum. Mol. Gen. 7, 2057-2062.

36. Ellies, L.G., Tsuboi, S., Petryniak, B., Lowe, J.B., Fukuda, M., and Marth, J.D. (1998).
Core 2 O-glycan biosynthesis distinguishes between selectin ligands essential for leukocyte homing and inflammation.
Immunity 9, 881-890.

37. Marek, K.W., Vijay, I., and Marth, J.D. (1999). A recessive deletion in the GlcNAc-1 phosphotransferase gene results in peri-implantation embryonic lethality. *Glycobiology* 9, 1263-1271.

38. Priatel, J.J., Chui, D., Hiraoka, N., Simmons, C.J.T., Richardson, K.B., Page, D.M., Fukuda, M., Varki, N.M., and Marth, J.D. (2000). The ST3Gal-I sialyltransferase controls CD8+ T cell homeostasis by modulating O-glycan biosynthesis. *Immunity* 12, 273-283.

39. Shafi, R., Iyler, S.P.N., O'Donnell, N., Ellies, L.G., Marek, K.W., Chui, D., Hart, G.W., and Marth, J.D. (2000). The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. *Proc. Natl. Acad. Sci. USA* 97, 5735-5739.

40. Marszalek, J.R., Liu, X., Roberts, L., Chui, D., Marth, J.D., Williams, D.S., and Goldstein, L.S.B. (2000). Genetic evidence for a kinesin-II mediated pathway for opsin transport in mammalian photoreceptors. *Cell* 102, 175-187.

41. Manzi, A.E., Norgard-Sumnicht, K., Argade, S., Marth, J.D., van Halbeek, H., and Varki, A.. (2000). Exploring the glycan repertoire of genetically modified mice by isolation and profiling of the major glycan classes and nano-NMR analysis of glycan mixtures. *Glycobiology* 10, 669-689.

42. Mak, T.W., Hakem, A., McPherson, J.P., Shehabeldin, A., Zablocki, E., Migon, E., Duncan, G.S., Bouchard, D., Wakeham, A., Cheung, A., karaskova, J., Sarosi, I., Squire, J., Marth, J., and Hakem, R. (2000). Brca1 required for T cell lineage development but not TCR loci rearrangement. *Nat. Immunol.* 1, 77-82.

43. Chui, D., Sellakumar, G., Green, R.S., Sutton-Smith, M., McQuistan, T., Marek, K.W., Morris, H.R., Dell, A., and Marth, J.D. (2001). Genetic re-modeling of protein glycosylation in vivo induces autoimmune disease. *Proc. Natl. Acad. Sci. USA* 98, 1142-1147.

44. DeFalco, J., Tomishima, Liu, H., M., Zhao, C., Marth, J.D., Enquist, L. and Friedman, J. (2001). Neural tracing with a conditional herpes virus: Mapping of inputs to a feeding center in the hypothalamus. *Science*, 291, 2608-2613.

45. Zhu, Y., Romero, M., Ghosh, P., Charnay, P., Rushing, E.J., Marth, J.D., and Parada, L.F. (2001). Ablation of NF1 function in neurons induces abnormal development of cerebral cortex and reactive gliosis in the CNS and PNS. *Genes and Development* 15, 859-876.

46. Snapp, K.R., Heitzig, C.E., Ellies, L.G., Marth, J.D., and Kansas, G.S. (2001). The O-linked branching enzyme core 2 β1-6 N-glucosaminyltransferase (C2 GlcNAcT-I (L)) is not essential for E-selectin ligand biosynthesis. *Blood* 97, 3806-3811.

47. Sperandio, M., Thatte, A., Foy, D., Ellies, L.G., Marth, J.D., and Ley, K. (2001). Severe impairment of leukocyte rolling in venules of core 2 glucosaminyltransferase deficient mice.

Blood 97, 3812-3819.

48. Yeh, J.-C., Hiroaka, N., Petryniak, B., Nakayama, J., Ellies, L.G., Marth, J.D., Lowe, J.B., and Fukuda, M. (2001). Novel sulfated lymphocyte homing receptors and their control by a Core 1 extension β1,3 N-acetylglucosaminyltransferase.
Cell 105, 957-969.

49. Sperandio, M., Forlow, S.B., Thatte, J., Ellies, L.G., Marth, J.D., and Ley, K. (2001). Differential requirements for Core 2 glucosaminyltransferase for endothelial L-selectin ligand function in vivo.
J. Immunol. 167, 2268-2274.

50. Moody, A.M., Chui, D., Reche, P.A., Priatel, J.J., Marth, J.D., and Reinherz, E.L. (2001). Developmentally regulated glycosylation of the CD8αβ co-receptor stalks modulates ligand binding.
Cell 107, 501-512.

51. Wang, Y., Tan, J., Sutton-Smith, M., Ditto, D., Panico, M., Campbell, R.M., Varki, N.M., Long, J.M., Jaeken, J., Levinson, S.R., Wynshaw-Boris, A., Morris, H.R., Le, D., Dell, A., Schachter, H., and Marth, J.D. (2001). Modeling human Congenital Disorder of Glycosylation Type IIa in the mouse: Conservation of asparagine-linked glycan-dependent functions in mammalian physiology and insights into disease pathogenesis.
Glycobiology 11, 1051-1070.

52. Onami, T.M., Lin, M-Y., Page, D.W., Marth, J.D., Reynolds, S., Katayama, C.D., Irimura, T., Varki, A. and Hedrick, S.M. (2002). The generation of mice deficient for the macrophage galactose and N acetylgalactosamine specific lectin: minimal role in lymphoid and erythroid homeostasis.
Mol. Cell. Biol. 22, 5173-5181.

53. Ellies, L.E., Ditto, D., Levy, G.G., Wahrenbock, M., Ginsburg, D., Varki, A., Le, D., and Marth, J.D. (2002). Sialyltransferase ST3Gal-IV operates as a dominant modifier of hemostasis by concealing asialoglycoprotein receptor ligands.
Proc. Natl. Acad. Sci. USA 99, 10042-10047.

54. Collins, B.E., Blixt, O., Bovin, N.V., Danzer, C.-P., Chui, D., Marth, J.D., Nitschke, L., and Paulson, J.C. (2002). Constitutively unmasked CD22 on B cells of ST6Gal-I knock-out mice: Novel sialoside probes for murine CD22.
Glycobiology, 12, 563-571.

55. Martin, L.T., Marth, J.D., Varki, A. and Varki, N.M. (2002). Genetically altered mice with different sialyltransferase deficiencies show tissue-specific alterations in sialylation and sialic acid 9-O-acetylation.
J. Biol. Chem. 277, 32930-32938.

56. Mertsching, E., Wurster, A.L., Katayama, C., Esko, J., Ramsdell, F., Marth, J.D., and Hedrick, S.M. (2002). A mouse strain defective for αβ vs. γδ T cell lineage commitment.
Int. Immunol. 14, 1039-1053.

57. Ellies, L.G., Sperandio, M., Underhill, G.H., Yousif, J., Smith, M., Priatel, J.J., Kansas, G.S., Ley, K., and Marth, J.D. (2002). Sialyltransferase specificity in selectin ligand formation.
Blood 100, 3618-3625.

58. Zhang, Y., Iwasaki, H., Wang, H., Kudo, T., Kalka, T.B., Hennet, T., Kubota, T., Cheng, L., Inaba, N., Gotoh, M., Togayachi, A., Guo, J., Hisatomi, H., Nakajima, K., Nishihara, S., Nakamura, M., Marth, J.D., and Narimatsu, H. (2003). Cloning and characterization of a new human UPD-*N*-acetyl- α-D-galactosamine: Polypeptide *N*-acetylgalactosaminyltransferase, designated ppGalNAcT-13, that is specifically expressed in neurons and synthesizes GalNAc α-serine/threonine antigen.
J. Biol. Chem. 278, 573-584.

59. Moody, A.M., North, S.J., Reinhold, B., Van Dyken, S., Rogers, M.E., Panico, M., Dell, A., Morris, H.R., Marth, J.D., and Reinherz, E.L. (2003). Sialic acid capping of CD8β Core 1 O-glycans controls thymocyte-MHCI interaction.
J. Biol. Chem. 278, 7240-7246.

60. Hiraoka, N., Kawashima, H., Petryniak, B., Nakayama, J., Mitoma, J., Marth, J.D., Lowe, J.B., and Fukuda, M. (2004). Core 2 branching β1,6-N-Acetylglucosaminyltransferase and HEV-restricted sulfotransferase collaboratively control lymphocyte homing.
J. Biol. Chem., 279, 3058-3067.

61. O'Donnell, N., Zachara, N., Hart, G.W., and Marth, J.D. (2004). Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability.
Mol. Cell. Biol., 24, 1680-1690.

62. Collins, B.E., Blixt, O., DeSieno, A.R., Bovin, N., Marth, J.D., and Paulson, J.C. (2004). Masking of CD22 by *cis* ligands does not prevent redistribution of CD22 to sites of cell contact.
Proc. Natl. Acad. Sci. USA, 101, 6104-6109.

63. Ye, Z. and Marth, J.D. (2004). N-glycan branching requirement in neuronal and post-natal viability.
Glycobiology, 14, 547-558.

64. Zachara, N.E., O'Donnell, N., Cheung, W.D., Mercer, J.J., Marth, J.D., and Hart, G.W. (2004). Dynamic O-GlcNAc Modification of Nucleocytoplasmic Proteins in Response to Stress: A Survival Response of Mammalian Cells
J. Biol. Chem. 279, 30133-30142.

65. Angata, K., Long, J.M., Bukalo, O., Lee, W., Ditayev, A., Wynshaw-Boris, A., Schachner, M., Fukuda, M., and Marth, J.D. (2004). Sialyltransferase ST8Sia-II assembles a subset of polysialic acid that directs hippocampal axonal targeting and promotes fear behavior.
J. Biol. Chem. 279, 32603-32613.

66. May, P., Rohlmann, A., Bock, H.H., Zurhove, K., Marth, J.D., Schomburg, E.D., Noebels, J.L., Beffert, U., Sweatt, J.D., Weeber, E.J., and Herz, J. (2004). Neuronal LRP1 functionally associates with postsynaptic proteins and is required for normal motor function in mice.
Mol. Cell. Biol. 24, 8872-8883.

67. Shi, S., Williams, S.A., Seppo, A., Kurniawan, H., Chen, W., Ye, Z., Marth, J.D., and Stanley, P. (2004). Inactivation of the *Mgat1* gene in oocytes impairs oogenesis, but embryos lacking complex
and hybrid N-glycans develop and implant.
Mol. Cell. Biol., 24, 9920-9929.

68. Gauguet, J.M., Rosen, S., Marth, J.D., and von Andrian, U. Core 2 GlcNAcT-I and HEC-GlcNAc6ST exert differential control over B and T lymphocyte homing to peripheral lymph nodes.
Blood, 104, 4104-4112.

69. Underhill, G.H., Zisoulis, D.G., Kolli, K.P., Ellies, L.G., Marth, J.D., and Kansas, G.S. A crucial role for T-bet in selectin ligand expression in T helper 1 (Th1) cells.
Submitted.

70. Sperandio, M., Ellies, L.G., Olson, T., Smith, M.L., Fritzsching, B., Pauly, E., Smith, D., Nobiling, R.,
Linderkamp, O., Marth, J.D., and Ley, K. L-selectin ligand function is differentially affected in lymphoid and non-lymphoid endothelial compartments of α2,3-sialyltransferase-IV deficient mice.
Submitted.

71. Brennan, P.J., Saouaf, S.J., Van Dyken, S., Marth, J.D., Li, B., Bhandoola, A., and Greene, M.I. Sialylation regulates peripheral tolerance in CD4+ T cells. Submitted.

72. Weinhold, B., Seidenfaden, R., Muhlenhoff, M., Schertzinger, F., Conzelmann, S., Rockle, I., Marth, J.D., Gerardy-Schahn, R., and Hildebrandt, H. Control of NCAM functions by polysialic acid is essential for brain wiring and post-natal development. Submitted.

73. Angata, K., Ranscht, B., Terskikh, A., Marth, J.D., and Fukuda, M. Polysialic acid-directed neural cell migration is required for brain development. Submitted.

74. Grewal, P., Boton, M., Rameriz, K., Collins, B.E., Chui, D., Paulson, J.C., and Marth, J.D. ST6Gal-I constructs and masks ligands for B cell and macrophage lectins that contribute to humoral immunity and autoimmune disease. Submitted.

75. Ohtsubo, K., Takamatsu, S., Minowa, M.T., Yoshida, A., Takeuchi, M., and Marth, J.D. Dietary regulation of pancreatic glucose transporter glycosylation is essential for glucose-stimulated insulin secretion in suppressing the pathogensis of diabetes. Submitted.

76. Tenno, M., Ohtsubo, K., Hagen, F., Tabak, L., and Marth, J.D. Selective contribution to selectin ligand formation by polypeptide GalNAcT-1 distinctly modulates leukocyte trafficking and function. Submitted.

77. Zheng, B., Tang, T., tang, N., Kudlicka, K., Ohtsubo, K., Ma, P., Marth, J.D., Farquhar, M.G., and Lehtonen, E. Essential role of RGS-PX1/sorting Nexin 13 in mouse development and organization of endocytic compartments. Submitted.

Reviews, Commentaries and Book Chapters:

1. Perlmutter, R.M., Marth, J.D., Ziegler, S.F., Garvin, A.M., Pawar, S., Cooke, M.P., and Abraham, K.M. (1988). Specialized protein tyrosine kinase proto-oncogenes in hematopoietic cells.
Biochem. Biophys. Acta. 948, 245-262.

2. Marth, J.D., Ong, C.J., and Chui, D. (1994). Specific CD45 isoforms regulate T cell ontogeny and are functionally distinct in modifying immune activation.
Adv. Exp. Med. Biol. 365, 149-166.

3. Marth, J.D. (1994). Will the transgenic mouse serve as a Rosetta Stone to glycoconjugate function?
Glycoconjugate J. 11, 3-8.

4. Varki, A. and Marth, J.D. (1995). Oligosaccharides in vertebrate development.
Sem. Dev. Biol. 6, 127-138.

5. Marth, J.D. (1996). Recent advances in gene mutagenesis by site-directed recombination.
J. Clin. Invest. 97, 1999-2002.

6. Marth, J.D. (1996). Complexity in mammalian O-linked oligosaccharide biosynthesis engendered by multiple polypeptide *N*-acetylgalactosaminyltransferases.
Glycobiology 6, 701-705.

7. Marth, J.D. (1999). N-Glycans.
In: *Essentials of Glycobiology* (Varki, Cummings, Esko, Freeze, Hart, Marth; eds.)
Cold Spring Harbor Press, N.Y., pp. 85-100.

8. Marth, J.D. (1999). O-Glycans.
In: *Essentials of Glycobiology* (Varki, Cummings, Esko, Freeze, Hart, Marth; eds.)
Cold Spring Harbor Press, N.Y., pp. 101-113.

9. Lowe, J.B. and Marth, J.D. (1999). Structures common to different types of glycans.
In: *Essentials of Glycobiology* (Varki, Cummings, Esko, Freeze, Hart, Marth; eds.)
Cold Spring Harbor Press, N.Y., pp. 211-252.

10. Marth, J.D. (1999). Determining the function of glycosylation using the genetically modified mouse.
In: *Essentials of Glycobiology* (Varki, Cummings, Esko, Freeze, Hart, Marth; eds.)
Cold Spring Harbor Press, N.Y., pp. 499-514.

11. Marth, J.D. (1999). Glycosylation changes in ontogeny and cell activation.
In: *Essentials of Glycobiology* (Varki, Cummings, Esko, Etzler, Freeze, Hart, Marth; eds.)
Cold Spring Harbor Press, N.Y., pp. 515-536.

12. Fukuda, M. and Marth, J.D. (2002).
ST3Gal-I: Balancing between core 1 and core 2 O-glycan biosynthesis.
In: *Handbook of Glycosyltransferases and their Related Genes* (Taniguchi, Honke, and Fukuda; eds.)
Springer-Verlag, N.Y., pp.267-273.

13. Wang, Y., Schachter, H., and Marth, J.D. (2002).
Mice with a homozygous deletion of the *Mgat2* gene encoding UDP-N-acetylglucosamine:

α-6-D-mannoside β-1,2-N-acetylglucosaminyltransferase II: A model for Congenital Disorder of Glycosylation (CDG) Type IIa.
Biochim. Biophys. Acta, 1573, 301-311.

14. Lowe, J.B., and Marth, J.D (2003).
A genetic approach to mammalian glycan function.
Ann. Rev. Biochem. 72, 673-691.

15. Marth, J.D. (2004).
Unmasking Connections in Transmembrane Immune Signaling.
Nat. Immunol. 5, 1008-1010.

Numerous Abstracts Published and Unpublished (1985-present)

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☑ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.